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THE NUTRITIONAL ECOLOGY
OF GREAT LAKES
CLADOPHORA GLOMERATA

by

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Various bioassays, primarily plant analysis, were utilized to evaluate relative nutrient supplies and primary growth limiting nutrients for Cladophora glomerata growth in parts of Green Bay, Lake Michigan, known to differ markedly in degree of pollution. Preliminary studies indicated emphasis should be on evaluations of five nutrients: phosphorus, nitrogen, boron, sulfur, and vitamin B_1. The bioassays indicated that phosphorus very likely is the critical nutrient in nuisance C. glomerata growths and that at times phosphorus supply actually is reduced to growth-limiting concentrations. However, the possibility that vitamin B_1 may at times be critical to C. glomerata cannot as yet be eliminated.

The bioassays employed (total P, hot-water-extractable P, and alkaline phosphatase activity) were in agreement and consistent in indicating phosphorus availability limited C. glomerata growth.

In further development of plant analysis as a bioassay, nitrogen and phosphorus critical concentrations were demonstrated to be relatively constant in C. glomerata of different ages and grown under various environmental conditions which would affect the rate and amount of growth.

A requirement for vitamin B_{12} and very high requirements for sulfur and boron were confirmed as unusual nutritional features of C. glomerata.

Data obtained from solution replacement cultures indicated that, even when adequate total phosphorus is available, the growth of C. glomerata becomes less than optimum at phosphorus solution concentrations of 0.014 ppm and less, and at potassium and calcium concentrations below 0.18 and 0.24 ppm, respectively.

Various algae, primarily from Lake Michigan, failed to produce inhibitors of C. glomerata growth to a degree that would suggest they are important in the ecology of C. glomerata in the Great Lakes.

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FOREWORD

Pollution from municipalities and industries has affected the Great Lakes adversely with increased growths of nuisance algae, particularly Cladophora glomerata, as one pollution response. Great masses of these algae, attached to rocks, floating on the surface, or blown onto swimming beaches, have become all too common in the Great Lakes.

While the correlation between nuisance Cladophora growths and pollution is obvious, the development of specific and effective control measures will be most efficiently carried out if the nutritional requirements of Cladophora glomerata are understood and if the key nutritional factors in pollutants can be identified. For a number of years, the laboratory of Dr. G. C. Gerloff in the Department of Botany at the University of Wisconsin-Madison has been involved in (1) laboratory studies on the nutrition of nuisance aquatic plants, (2) application of the nutritional data in the control of nuisance growths, and (3) development of plant analysis as a reliable procedure for evaluating nutrient supplies in aquatic ecosystems.

This is a report on recent studies of the laboratory on Cladophora glomerata, with essentially the objectives mentioned above. The work has progressed to the point that practical applications are possible, for example in the conclusion that phosphorus is the critical factor in the development of nuisance Cladophora growths. This makes possible the development of control measures. based on reduction of phosphorus input, with confidence that the measures will be effective.

Michael S. Adams
Professor of Botany
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Plant Ecology

ABSTRACT

Various bioassays, primarily plant analysis, were utilized to evaluate relative nutrient supplies and primary growth limiting nutrients for *Cladophora glomerata* growth in parts of Green Bay, Lake Michigan, known to differ markedly in degree of pollution. Preliminary studies indicated emphasis should be on evaluations of five nutrients: phosphorus, nitrogen, boron, sulfur, and vitamin B_1. The bioassays indicated that phosphorus very likely is the critical nutrient in nuisance *C. glomerata* growths and that at times phosphorus supply actually is reduced to growth-limiting concentrations. However, the possibility that vitamin B_1 may at times be critical for *Cladophora glomerata* cannot as yet be eliminated.

The bioassays employed (total P, hot-water-extractable P, and alkaline phosphatase activity) were in agreement and consistent in indicating phosphorus availability limited *C. glomerata* growth.

In further development of plant analysis as a bioassay, nitrogen and phosphorus critical concentrations were demonstrated to be relatively constant in *Cladophora glomerata* of different ages and grown under various enviornmental conditions which would affect the rate and amount of growth.

A requirement for vitamin B_{12} and very high requirements for sulfur and boron were confirmed as unusual nutritional features of *Cladophora glomerata*. Comparisons with the requirements for other green and blue-green algae established the uniqueness of the high sulfur requirement for

C. glomerata. There was no indication of sulfur deficiency in *Cladophora* sp. from the Great Lakes.

Date obtained from solution replacement cultures indicated that, even when adequate total phosphorus is available, the growth of *Cladophora glomerata* becomes less than optimum at phosphorus solution concentrations of 0.014 ppm and less, and at potassium and calcium concentrations below 0.18 and 0.24 ppm, respectively. This supports the concept of critical concentrations in the water for optimum *C. glomerata* growth.

Trace element studies indicated *Cladophora glomerata* requires an inorganic nutrient not generally recognized as essential for plant growth. The alga responded positively and significantly to a mixture of elements of suspected essentiality. However, systematic elimination of elements from the mixture failed to identify one element as responsible for the yield increases. The situation is more complex than anticipated.

The culture and nutrition of *Nitzschia palea*, a very common diatom in the Great Lakes, was investigated. Silicon concentrations well above those in available media were essential for successful laboratory growth of *Nitzschia palea*.

Various algae, primarily from Lake Michigan, failed to produce inhibitors of *C. glomerata* growth to a degree that would suggest they are important in the ecology of *Cladophora glomerata* in the Great Lakes.

Laboratory studies were initiated on the toxicity of heavy metals to *Cladophora glomerata*.

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to September 30, 1978, and work was completed as of September 29, 1978.

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SECTION 1

INTRODUCTION

The pollution of the Great Lakes with municipal and industrial wastes has resulted in increased growths of nuisance algae. Filamentous green algae in the genus Cladophora are among the most troublesome of the nuisance organisms. The development of measures for the reduction of the Cladophora growths, based on nutrient limitation, will be facilitated by an understanding of the importance of specific nutrients in the growth of Cladophora in the field, in other words, in the nutritional ecology of the Cladophora. A primary goal of this project has been to identify the nutrients, both inorganic and organic, most likely to limit and control the growth of Cladophora glomerata in Lake Michigan.

The knowledge of the qualitative and quantitative nutritional requirements necessary to evaluate the field nutrition of Cladophora glomerata usually can be obtained most effectively in the laboratory. An earlier report (Gerloff and Fitzgerald, 1976) described successful efforts to bring Cladophora sp. into laboratory culture in a synthetic medium and to study its nutrition in detail. Requirements for vitamins B_1 and B_{12} and very high requirements for boron (B) and sulfur (S) were among the unusual nutritional features of Cladophora glomerata that were identified. Additional nutritional studies will be presented in this report, particularly studies on nutritional features which might be significant in nuisance Cladophora growths in

Lake Michigan.

The bioassay known as plant or tissue analysis has been the primary procedure used on this project to evaluate the nutrition of Cladophora sp. in the Great Lakes and to identify the nutrients most likely to control excessive growths. The initial results of plant analysis, described in an earlier report (Gerloff and Fitzgerald, 1976), suggested phosphorus (P) to be the primary limiting element. However, this conclusion was based on relatively few samples. It seemed worthwhile therefore to extend the survey by more thoroughly sampling a specific area in Lake Michigan and by verifying plant analysis results with results from other bioassays. One such bioassay involved a lake water enrichment technique similar to the Algal Assay Procedure (National Eutrophication Research Program, 1971). Specific bioassays for P included total tissue P, hot-water extractable P (Fitzgerald, 1969), and a procedure based on the activity of the enzyme alkaline phosphatase (Fitzgerald and Nelson, 1966). Total N, uptake of NH_4-N in the dark (Fitzgerald, 1969) and accumulation of NO_3-N in the algae (Ulrich, 1961; Cataldo, et al, 1975) were tested as bioassays for available N. Bioassays based on algae analyses and growth avoid many problems of sampling and data interpretation associated with chemical analyses of water samples (Gerloff, 1969; Gerloff, 1973; Gerloff and Fishbeck, 1973).

Studies on the primary growth limiting nutrients for Cladophora glomerata, and other nuisance aquatic organisms, seem justified even though it is generally agreed that reduction in P input usually is the most practical procedure for a nutritional control program. However, it cannot be assumed that P always is the key limiting nutrient in nuisance algae growths (Ryther and Dunstan, 1971; Gerloff, 1975). This is particularly true for an organism such as Cladophora which requires external supplies of organic factors, vitamins B_1 and B_{12}, as well as inorganic. The presence of the vitamins in organic pollutants or their production by bacteria could be critical in Cladophora growth. Also, reliable and simple assays are needed to predict the degree of success of control measures based on reduced P input. It cannot be assumed that every reduction in the level of P in a body of water will reduce nuisance aquatic growths, because the element may not be reduced to a limiting level. Assays which would determine whether P, or some other nutrient, limits Cladophora growth in a specific area at a specific time would be useful.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The conclusions from this project can most readily be evaluated when related to the primary objectives. Those goals were (1) to identify the nutrient which is the key or limiting factor in the development of nuisance _Cladophora glomerata_ growths in Lake Michigan and (2) to continue laboratory experiments on aspects of the nutrition of _Cladophora glomerata_ which will be useful in understanding the ecology of that organism.

The primary conclusions are:

1. _Cladophora glomerata_ becomes phosphorous deficient in the least polluted parts of Green Bay during late summer. Phosphorus seems to be the key nutrient in the development of nuisance _C. glomerata_ growths.
2. There is a close correlation between pollution of Green Bay and the development of phosphorus deficiency in _Cladophora glomerata_.
3. _Cladophora glomerata_ has extremely high and unique requirements for sulfur and boron, but these elements are not critical in the nutritional ecology of the organism.
4. While phosphorus very likely is the key factor in nuisance _Cladophora glomerata_ growths, the possibility that vitamin B_1 is a critical nutrient cannot be eliminated at this time.
5. Relatively simple bioassays are available for evaluating nutrient supplies for _Cladophora glomerata_.

6. Critical nitrogen and phosphorus concentrations, which are the basis of nutrient assays through plant analysis, are relatively constant for Cladophora glomerata under a variety of environmental conditions and at various stages of growth. This is a critical point of support for plant analysis as a reliable nutrient bioassay.
7. Cladophora glomerata growth seems to be less than optimal when the phosphorus solution concentration decreases to 0.014 ppm and below.
8. Inhibitor production by common algae probably is not a major factor in the ecology of Cladophora glomerata.
9. An adequate supply of soluble silicon is a critical factor in the successful laboratory culture of the diatom Nitzschia palea. Media commonly used for culturing blue-green and green algae contain inadequate silicon for optimum growth of the diatom.

The primary recommendations which seem justified from the information obtained are:

1. Reduction of the phosphorus input probably is an effective means of reducing nuisance Cladophora glomerata growths in the Great Lakes and should be implemented.
2. Further studies should be carried out on the vitamin B_1 field nutrition of Cladophora glomerata in the Great Lakes and on the possible key role of vitamin B_1 in nuisance growths under some conditions.
3. Plant analysis and other bioassays should be more widely used in evaluating nutrient supplies and critical nutrients for nuisance growths of Cladophora glomerata and other species.
4. Further laboratory studies on the trace element nutrition, particularly heavy metal toxicity, of Cladophora glomerata, and the other primary

algae of the Great Lakes, should be carried out and the results should be related to the ecology of the algae in the Great Lakes.

SECTION 3

CONTROLLING NUTRITIONAL FACTORS IN NUISANCE *Cladophora glomerata* GROWTHS IN LAKE MICHIGAN

The results of various bioassays to identify the critical or controlling nutrients for nuisance growths of *Cladophora glomerata* are presented in this section. Primary emphasis is on "plant analysis" as a diagnostic technique, partly because of the long-term interests of the Principal Investigator in this procedure and also because it indicated a close correlation between pollution and the algae concentrations of N and P on samples of *Cladophora* sp. randomly collected from the Great Lakes (Gerloff and Fitzgerald, 1976). This was particularly apparent in samples from Green Bay (Fitzgerald, Torrey, and Gerloff, 1975) in which the P concentration in *Cladophora* sp. progressively decreased with increasing distance between the collection site and the point of entry of the nutrient-rich Fox River into lower Green Bay. Green Bay seemed a particularly desirable area to sample in further studies.

The initial studies on nutrient supplies in Green Bay, involved samples collected only on one date. It seemed worthwhile to sample algae at specific sites several times during the growing season. Also, because Cladophora glomerata was shown to have very high requirements for S and B, these elements, in addition to N and P, were considered possible key nutrients, and were included in the plant analysis assay.

GENERAL EXPERIMENTAL PROCEDURES

For the bioassays, samples of *C. glomerata* and of lake water were obtained primarily at five Lake Michigan sites (Figure 1). Four of the sites were on the Green Bay side of Door County; the fifth site was on the Lake Michigan side. The sites were selected to represent a range of pollution and fertility, resulting primarily from the entry of the Fox River into lower Green Bay.

Statistical analyses were run on the dry-weight algae yields from the experiments included in this and other sections of the report. Homogenicity of variance within an experiment was tested using Bartlett's and F_{max}-tests (Sokal and Rohlf, 1969). If the variances of the treatments were not homogeneous, these tests were used to create homoscedastic groups within an experiment. Homoscedastic groups were then tested individually to determine significant difference at the 5% level using the Student-Newman-Keuls (SNK) multiple range test (Sokal and Rohlf, 1969). An approximate test, the equality of means with heterogeneous variances (EMHV), was used to indicate significant difference at the 5% level between non-homogeneous or heteroscedastic groups (Sokal and Rohlf, 1969).

C. glomerata samples were obtained by clipping filaments, indicated as healthy by green color, from heavy growths attached to rocks close to the shore or from rock-breakwaters which extended out from the shore. The algae samples were repeatedly rinsed in distilled water to remove attached algae and other obvious, contaminating debris. After rinsing, as much water as possible was hand-squeezed from the algae. The algae then were placed in nylon bags and transported to the laboratory under refrigeration. The algae were oven-dried at 65C and ground in a Wiley Mill. Analyses for N, P, S, and B were by generally accepted quantitative techniques. Unfortunately, all of the samples from a June sampling were lost because of the failure of a laboratory deep-freeze unit.

PLANT ANALYSIS

In the plant analysis bioassay, reduction in the supply of an element to a growth-controlling level for a particular species at a particular site is indicated by a concentration of the element in a sample of the species at or below the critical concentration, the minimum plant concentration which permits optimum growth. A concentration above the critical level indicates the algae from the site sampled were adequately supplied with the element. The dates of Cladophora glomerata sampling and the results of algae analyses for N, B, S, and P are presented in Figures 1-4, respectively.

The critical N concentration for C. glomerata is 1.10% (Gerloff and Fitzgerald, 1976). It is apparent from Figure 1 that no N concentration was close to that value. The lowest concentration was 2.00% in the August 15 sample from Bayshore Park; all other values were more than double the critical concentration. There was no indication that C. glomerata became N

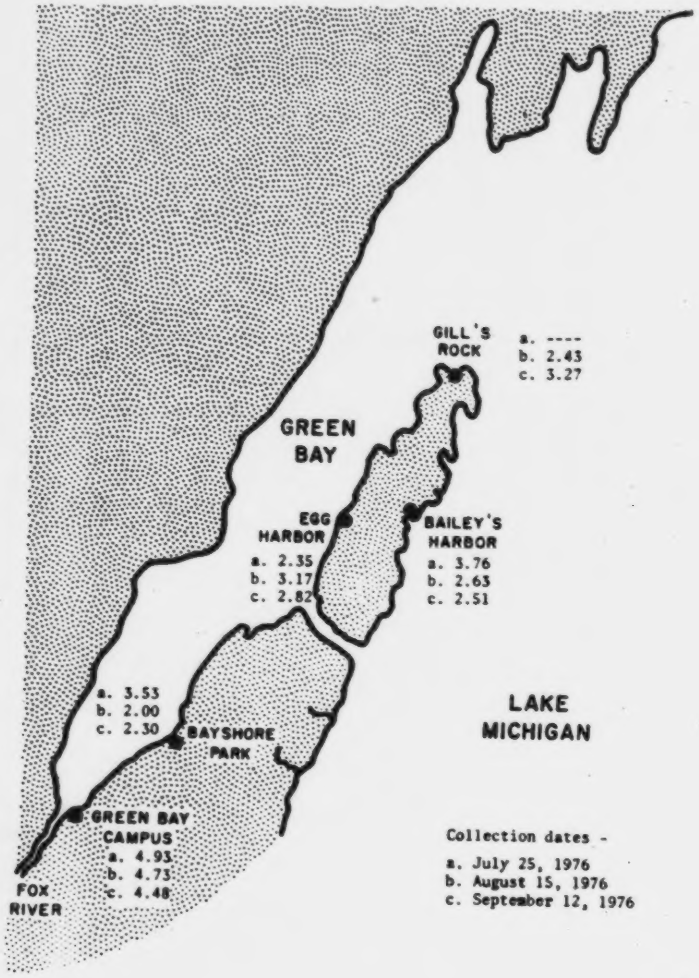
GILL'S ROCK
a. ----
b. 2.43
c. 3.27
GREEN BAY
EGG HARBOR
a. 2.35
b. 3.17
c. 2.82
BAILEY'S HARBOR
a. 3.76
b. 2.63
c. 2.51
a. 3.53
b. 2.00
c. 2.30
BAYSHORE PARK
LAKE MICHIGAN
GREEN BAY CAMPUS
a. 4.93
b. 4.73
c. 4.48
FOX RIVER
Collection dates -
a. July 25, 1976
b. August 15, 1976
c. September 12, 1976

Figure 1. Total Nitrogen (%) in Cladophora

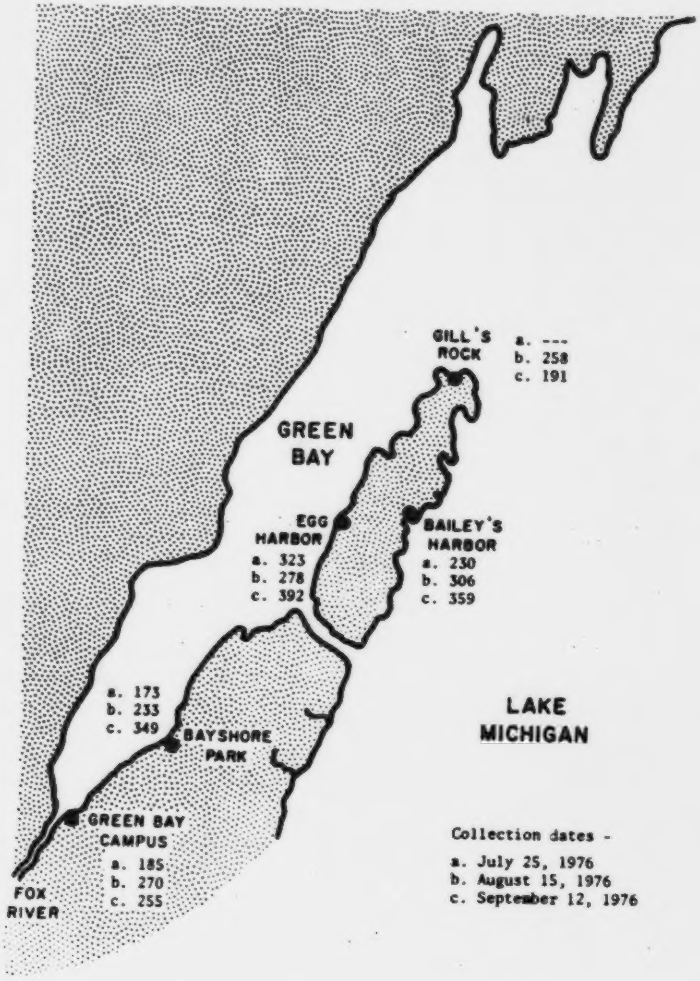
GILL'S ROCK
a. ---
b. 258
c. 191
GREEN BAY
EGG HARBOR
a. 323
b. 278
c. 392
BAILEY'S HARBOR
a. 230
b. 306
c. 359
a. 173
b. 233
c. 349
BAYSHORE PARK
LAKE MICHIGAN
GREEN BAY CAMPUS
a. 185
b. 270
c. 255
FOX RIVER
Collection dates -
a. July 25, 1976
b. August 15, 1976
c. September 12, 1976

Figure 2. Boron (ppm) in Cladophora

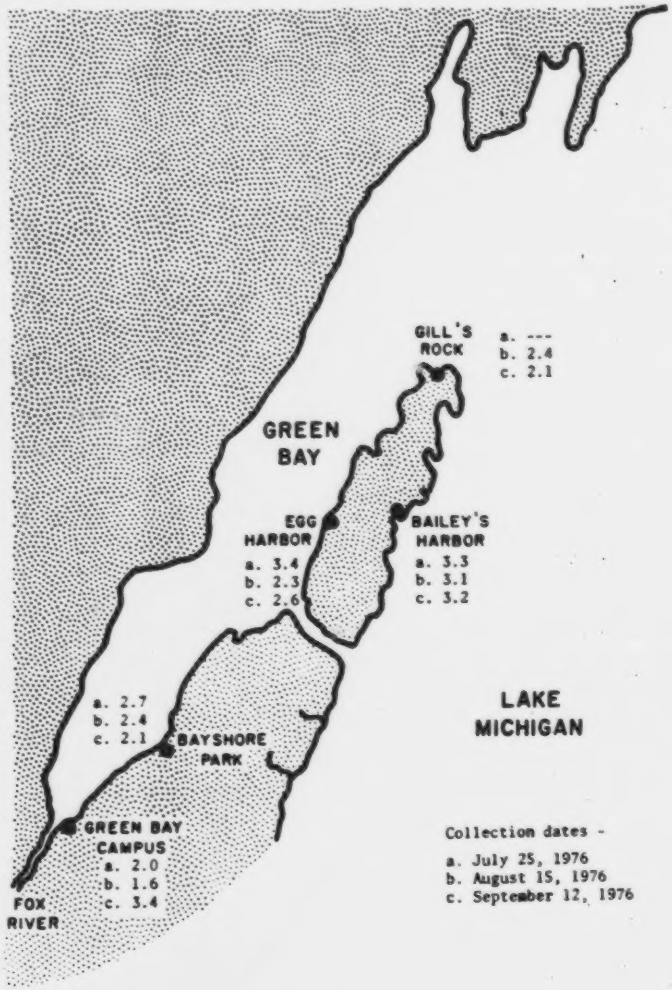
GILL'S ROCK
a. ---
b. 2.4
c. 2.1
GREEN BAY
EGG HARBOR
a. 3.4
b. 2.3
c. 2.6
BAILEY'S HARBOR
a. 3.3
b. 3.1
c. 3.2
a. 2.7
b. 2.4
c. 2.1
BAYSHORE PARK
LAKE MICHIGAN
GREEN BAY CAMPUS
a. 2.0
b. 1.6
c. 3.4
FOX RIVER
Collection dates -
a. July 25, 1976
b. August 15, 1976
c. September 12, 1976

Figure 3. Sulfur (%) in Cladophora

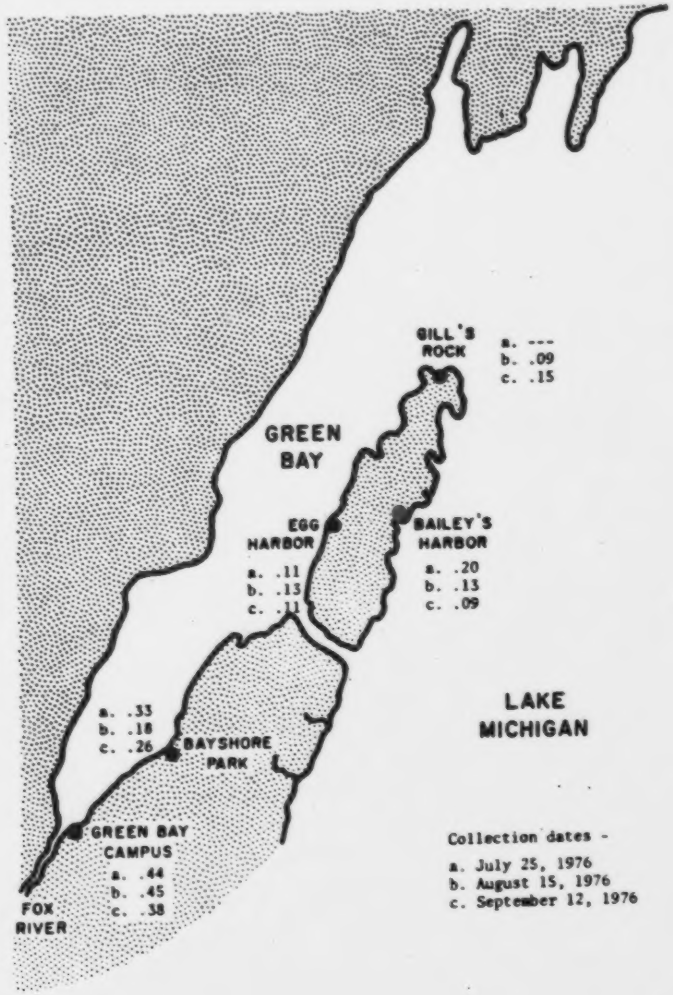
GILL'S ROCK
a. ---
b. .09
c. .15
GREEN BAY
EGG HARBOR
a. .11
b. .13
c. .11
BAILEY'S HARBOR
a. .20
b. .13
c. .09
a. .33
b. .18
c. .26
BAYSHORE PARK
LAKE MICHIGAN
GREEN BAY CAMPUS
a. .44
b. .45
c. .38
FOX RIVER
Collection dates -
a. July 25, 1976
b. August 15, 1976
c. September 12, 1976

Figure 4. Total Phosphorus (%) in Cladophora

deficient at any of the five sample areas during July, August, and September. The high N concentrations in the samples from the University of Wisconsin Campus at Green Bay correlate with the entrance of pollution at the lower end of Green Bay via the Fox River.

The critical B concentration in C. glomerata is 110 ppm. As with N, there was no indication that B supply became growth-limiting at the sample sites (Figure 2). The lowest B concentration was 173 ppm in the July 25, Bayshore Park sample; most values were at least double the critical concentration.

Relatively, S concentrations in the samples were closer to the critical value of 1.5% than were the N and B concentrations (Figure 3). In fact, the 1.6% S concentration in the August 15 sample was almost at the critical concentration. The plant analysis results, in general, did not indicate S supply was critical for C. glomerata growth in Green Bay. It seems interesting that the two lowest algae S concentrations were in samples from the most eutrophic part of Green Bay.

The analyses for total P in the C. glomerata samples are presented in Figure 4. One interesting aspect of the P data is the correlation between the degree of pollution of Green Bay and the P concentration in the algae. The average P concentration in the three Green Bay Campus samples was 0.42%; in the Bayshore Park samples, 0.26%; and in the samples from the other three sites, 0.13%.

No C. glomerata P concentration was as low as the 0.08% critical concentration. However, in seven of the samples the P concentration was below 0.15% and in two of the samples was only 0.09%. In an earlier phase of this project, the plant analysis technique indicated that P did become a growth-limiting nutrient in the upper portions of Green Bay (Fitzgerald, Torrey, and Gerloff, 1975; Gerloff and Fitzgerald, 1976).

DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE WATER SAMPLES

Lake water samples were collected at the same sites as *Cladophora glomerata* and immediately were packed in ice for transport to the laboratory. After filtering through Reeve-Angel 934AH glass fibre filters, the samples were frozen and kept in that condition until utilized in experiments.

Aliquots of the lake water from specific sites were enriched with nutrients as indicated in Tables 1 to 5. Concentrations of added nutrients were those in the synthetic medium developed for *Cladophora* sp. culture on this project (Gerloff and Fitzgerald, 1976). All the essential inorganic and organic nutrients were added to lake water in Treatment B; Treatment A was the synthetic *Cladophora* medium prepared with distilled water. In the remaining treatments, one or several essential nutrients were omitted in each treatment. This approach made possible evaluations of the relative amounts of specific nutrients present in lake water samples.

The *C. glomerata* to be used as inoculum was transferred to a solution containing only Na_2CO_3 and Na_2SiO_3 for 48-72 hours prior to inoculation of an experiment. All cultures were incubated for 21 days under continuous fluorescent light of approximately 500 ft-c and at a temperature of 23 ± 1C. Details of procedures used for culturing and harvesting *Cladophora* can be found in Gerloff and Fitzgerald (1976).

In most tests, the cultures were autoclaved prior to inoculation. Because of concern over possible destruction of vitamins B_1 and B_{12} and precipation of iron (Fe) in non-available forms, several duplicate treatments were included in which the lake water was not autoclaved but passed through a Whatman GF/F filter. A control with all nutrients added to the filtered water also was prepared.

The results of the enrichment tests on water collected at the five Lake Michigan sites are presented in Tables 1 through 5. July and September water samples from all the sites were used in the enrichment tests. Because of insufficient time to prepare the experiments, the August water samples were used from only several sites. Due to its location on lower Green Bay, the sample site designated as Green Bay Campus - University of Wisconsin provided water relatively high in nutrients. This was supported by the appearance of heavy blue-green algae blooms, as well as Cladophora glomerata, at this location. The Bayshore Park site also was at a rather heavily polluted section of Green Bay. Egg Harbor, Gill's Rock, and Bailey's Harbor are in relatively unpolluted areas of Green Bay and Lake Michigan.

As anticipated, some differences in the results related to variations in water fertility, for example, the greater growth in the minus P treatment prepared with Green Bay Campus and Bayshore Park water. Nevertheless, for evaluating primary limiting nutritional factors, variations between sample sites were not significant.

Any element present in lake water in amounts adequate to support 50 per cent of the yield in the complete medium in lake water (Treatment B) was considered unlikely to limit algae growth under field conditions. Of the nutrients tested, this eliminated calcium (Ca), magnesium (Mg), manganese (Mn), molybdenum (Mo), zinc (Zn), copper (Cu), boron (B), sulfur (S) and vitamin B_{12} as critical in C. glomerata growth. The average yields of 72% of Treatment B in the minus S and 60% in the minus B cultures prepared in low B glassware are of particular interest because of the

TABLE 1. RESPONSE OF *Cladophora glomerata* TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT GREEN BAY CAMPUS

Designation	Treatment	Date sampled[†]			
		July 26, 1976		September 12, 1976	
		Ave. mg/liter	% of B	Ave. mg/liter	% of B
A	*Cladophora* medium*	562	71	584	94
B	*Clad*. medium in lake water	793	100	620	100
C	As B, - vit. B_1	82	10	132	21
D	As B, - vit. B_{12}	435	55	556	90
E	As B, - N	87	11	141	23
F	As B, - P	140	18	287	46
G	As B, - Fe	116	15	249	40
H	As B, - S	704	89	686	111
I	As B, - B, pyrex	818	103	-	-
J	As B, - B, low B glass	396	50	356	57
K	As B, - K	319	40	271	44
L	As B, - Ca, Mg, Mn, Mo, Zn, Cu	749	94	538	87
M	As B, but filtered l. w. + vit.	-	-	773	125
N	As M, - vit. B_1	-	-	82	13
O	As M, - vit. B_{12}	-	-	638	103
P	As M, - Fe	-	-	461	74

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 1.8 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 and C_{13} solutions, 4 ppm vitamin B_1, 0.5 µg/l vitamin B_{12} and Na_2CO_3 and Na_2SiO_3.

† Average of five replicates in each treatment except J, July, which has three.

very high requirements for those two elements. The enrichment tests gave no indication of a growth-limiting role for S and B.

Potassium (K) seemed appropriately designated as an element "unlikely to be limiting but should not be disregarded." Yield in the minus K treatment varied from 23% in the July 25 water from Bailey's Harbor to 44% in the September 12 Green Bay Campus sample.

Every enrichment test indicated that four nutrients (N, P, Fe, and vitamin B_1) were most likely to limit growth. The average yield in the -N treatment in the samples from the three least fertile sites (Egg Harbor, Gill's Rock, and Bailey's Harbor) was 7%; in the -P treatment, 15%; in the -Fe, 16%; and with vitamin B_1 omitted, 12%.

Some yield differences among the four most limiting nutrients were significantly different; others were not. For example Table A-8 in Appendix A shows that in the July 25 sample from Gill's Rock yield was significantly different in the minus P cultures compared to the minus N (heteroscedastic group VII) but minus P was not significantly different from minus vitamin B_1 or minus Fe. All evaluations and summaries of the enrichment data indicated N to be the most likely growth-limiting nutrient in the lake water samples.

TABLE 2, RESPONSE OF *Cladophora glomerata* TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT BAYSHORE PARK

		Date sampled†					
		July 26, 1976		August 15, 1976		September 12, 1976	
Designation	Treatment	Ave. mg/liter	% of B	Ave. mg/liter	% of B	Ave. mg/liter	% of B
A	*Cladophora* medium*	649	83	494	84	564	74
B	*Clad*. medium in lake water	778	100	586	100	761	100
C	As B, - vit. B_1	71	22	134	23	80	11
D	As B, - vit. B_{12}	324	42	706	120	525	69
E	As B, - N	52	7	73	12	94	12
F	As B, - P	164	21	177	30	190	25
G	As B, - Fe	155	16	124	21	102	13
H	As B, - S	501	64	-	-	476	63
I	As B, - B, pyrex	869	112	-	-	-	-
J	As B, - B, low B glass	420	54	338	58	430	56
K	As B, - K	280	36	-	-	240	32
L	As B, - Ca, Mg, Mn, Mo, Zn, Cu	709	91	-	-	254	33
M	As B, but filtered l.w. + vit.	-	-	718	123	543	71
N	As M, - vit. B_1	-	-	169	29	75	10
O	As M, - vit. B_{12}	-	-	-	-	458	60
P	As M, - Fe	-	-	-	-	70	9

* The nutrient medium is the same as that described in Table 1.

† Average of five replicates in each treatment except for J, July, which has three and D, August, which has two.

ADDITIONAL BIOASSAYS

Because no single test seems sufficiently reliable to permit firm conclusions on primary growth-limiting nutrients, several additional bioassays which have been proposed and tested for estimating N and P availability for aquatic plant growth (Fitzgerald and Nelson, 1966; Fitzgerald, 1969) were used to evaluate Lake Michigan nutrients. This included hot water extractable P and alkaline phosphatase activity as P assays and ammonium-nitrogen uptake in the dark and nitrate accumulation as N assays.

Borderline or critical concentrations had to be established which would distinguish between _C_. _glomerata_ adequately supplied with N or P and plants deficient in these elements. It could not be assumed that the values established for other aquatic plants would apply to _C_. _glomerata_.

As indicated in Figure B-1, and Table B-1 in Appendix B, the critical concentration for the Fitzgerald hot-water extractable P test was 0.013%. The alkaline phosphatase assay for P is based on the observation that increased alkaline phosphatase enzyme activity is induced in plant tissues under P deficiency. As a result, the more P deficient the environment from which plants are collected, the higher the alkaline phosphatase activity. The calibration test reported in Table B-2, and other unreported tests, indicated the threshold alkaline phosphatase level for _C_. _glomerata_, to be 150 units per mg of dried algae.

TABLE 3. RESPONSE OF *Cladophora glomerata* TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT EGG HARBOR

		Date sampled†			
		July 25, 1976		September 12, 1976	
Designation	Treatment	Ave. mg/liter	% of B	Ave. mg/liter	% of B
A	*Cladophora* medium*	597	90	690	90
B	*Clad*. medium in lake water	660	100	764	100
C	As B, - vit. B_1	107	16	76	10
D	As B, - vit. B_{12}	-	-	606	79
E	As B, - N	21	3	86	11
F	As B, - P	64	10	149	19
G	As B, - Fe	72	11	288	38
H	As B, - S	543	82	493	65
I	As B, - B, pyrex	713	108	-	-
J	As B, - B, low B glass	545	83	342	45
K	As B, - K	200	30	220	29
L	As B, - Ca, Mg, Mn, Mo, Zn, Cu	580	88	565	74
M	As B, but filtered l. w. + vit.	—	-	539	71
N	As M, - vit. B_1	-	-	44	6
O	As M, - vit. B_{12}	-	-	389	51
P	As M, - Fe	-	-	142	19

* The nutrient medium is the same as that described in Table 1.

† Average of five replicates in each treatment except for J, July, which has three.

The uptake of ammonium-nitrogen in the dark bioassay for N availability recognizes that as plants become N deficient, their capacity for NH_4-N uptake during a specific time interval increases (Fitzgerald, 1969). Nitrate also is known to accumulate in many species under conditions of high N supply. This has frequently been used as an assay for environmental N supply in agricultural and horticultural crops, for example by Ulrich (1961). Efforts to calibrate these two tests for Cladophora glomerata are reported in Tables B-3 and B-4 in Appendix B. Neither test was satisfactory. When cultured over a wide range of N supply in the nutrient medium, there was neither a marked increase in the rate of NH_4-N uptake nor an accumulation of NO_3-N in the critical zone in which Cladophora yields first reached a maximum with increasing external N supply. In other words, NH_4-N uptake and NO_3 accumulation were not sufficiently sensitive indicators of the changing N status of the Cladophora in the critical zone of yield change.

Results of three bioassays for P availability to C. glomerata collected from five Green Bay sites during July and August are presented in Table 6. No analysis of the July 11 samples indicated P was limiting algae growth. This is not surprising because July 11 is relatively early in the growing season, and nutrient supplies had not come under stress.

There was excellent correlation between the various assays on the samples taken August 24. All three tests indicated that Green Bay Campus

TABLE 4. RESPONSE OF *Cladophora glomerata* TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT GILL'S ROCK

		Date sampled[†]					
		July 25, 1976		August 15, 1976		September 12, 1976	
Desig-nation	Treatment	Ave. mg/liter	% of B	Ave. mg/liter	% of B	Ave. mg/liter	% of B
A	*Cladophora* medium*	840	81	635	76	742	100
B	*Clad*. medium in lake water	1038	100	829	100	741	100
C	As B, - vit. B_1	239	23	81	10	103	14
D	As B, - vit. B_{12}	566	55	381	46	665	90
E	As B, - N	43	4	130	16	66	9
F	As B, - P	181	17	43	5	185	25
G	As B, - Fe	152	15	85	10	81	11
H	As B, - S	458	44	473	57	665	90
I	As B, - B, pyrex	920	89	-	-	-	-
J	As B, - B, low B glass	578	56	415	50	742	100
K	As B, - K	350	34	228	27	231	31
L	As B, - Ca, Mg, Mn, Mo, Zn, Cu	712	69	866	105	582	79
M	As B, - Mn	-	-	784	95	-	-
N	As B, but filtered l.w. + vit.	-	-	680	82	806	109
O	As N, - vit. B_1	-	-	57	7	329	44
P	As N, - vit. B_{12}	-	-	-	-	631	85
Q	As N, - Fe	-	-	-	-	59	8

* The nutrient medium is the same as that described in Table 1.

† Average of five replicates in each treatment except D and H, September, which have 4, J and O, August, and J, July, which have three and N, August which has two.

and Bayshore Park algae were well supplied with P. In addition, all tests indicated the Egg Harbor C. glomerata was P deficient. Total P in the C. glomerata was at the critical total P concentration of 0.08%; the hot water extractable P was just below the 0.013% critical level; the alkaline phosphatase activity was at 191 which is considerably above the 150 threshold value. All three tests also indicated the Gill's Rock and Bailey's Harbor algae to be close to P deficiency.

Several of the bioassays were run on Cladophora glomerata, collected from six sites on September 18, 1977 (Table 7). This is near the end of the period of peak algae growth. The total N concentration in every sample was far above the 1.1% critical concentration. In contrast, the total P concentration in the Egg Harbor and Newport State Park samples was below the 0.08% critical level. The hot water extractable values for those two samples were very close to the 0.013% critical concentration. The algae were P-deficient. Both the total and hot water extractable values were much higher on the samples from the other four sites.

DISCUSSION

The primary result from the enrichment tests was the evidence that only four nutrients, N, P, Fe, and vitamin B_1, need be seriously considered as growth-limiting factors for nuisance Cladophora glomerata growths in Green Bay. Although the data are somehwat inconsistent, the results

TABLE 5. RESPONSE OF *Cladophora glomerata* TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT BAILEY'S HARBOR

		Date sampled[†]			
		July 25, 1976		September 12, 1976	
Designation	Treatment	Ave. mg / liter	% of B	Ave. mg / liter	% of B
A	*Cladophora* medium*	821	90	653	86
B	*Clad*. medium in lake water	912	100	763	100
C	As B, - vit. B_1	58	6	58	8
D	As B, - vit. B_{12}	720	79	433	57
E	As B, - N	42	5	58	8
F	As B, - P	84	9	156	20
G	As B, - Fe	146	16	84	11
H	As B, - S	593	65	505	66
I	As B, - B, pyrex	907	99	-	-
J	As B, - B, low B glass	584	64	346	45
K	As B, - K	213	23	201	26
L	As B, - Ca, Mg, Mn, Mo, Zn, Cu	543	60	465	61
M	As B, but filtered l. w. + vit.	-	-	388	51
N	As M, - vit. B_1	-	-	41	5
O	As M, - vit. B_{12}	-	-	123	16
P	As M, - Fe	-	-	8	1

* The nutrient medium is the same as that described in Table 1.

† Average of five replicates in each treatment except D, G, and L, July, and B, September, which have four and J, July, and P, September which have three.

further indicated that of those nutrients N was the most critical. In evaluating this result, it should be recognized that the enrichment procedure is subject to a source of error which may be responsible for errors in all assay procedures involving water samples withdrawn from a lake at a specific site and time. The nutrients no longer are replenished as they are utilized, for example, from organic matter decay and through release from bottom sediments. The failure of plant analysis to indicate even borderline N deficiency in any sample confirms the above source of error and emphasizes the need to translate the enrichment test data to field conditions with considerable caution.

Two general points relating to the culture and nutrition of C. glomerata were apparent from the results. First, the data from all sites showed that yields consistantly were slightly better when nutrients, in the concentrations normally found in the synthetic culture medium, were added to lake water rather than distilled water (Treatment A vs. B). This suggests that lake water contains an unrecognized essential trace nutrient or that the concentration ratios of nutrients in the synthetic medium are not optimal. Second, autoclaving lake water does not inactivate Fe or destroy vitamins B_1 and B_{12} to a degree that C. glomerata growth is affected. If inactivation or precipitation did occur, responses to the omission of the vitamins and Fe would have been greater in autoclaved than in filtered lake water.

TABLE 6. BIOLOGICAL ASSAYS FOR ADEQUACY OF P SUPPLY FOR *Cladophora glomerata* GROWTH AT VARIOUS GREEN BAY AND LAKE MICHIGAN SITES

	July 11, 1977			August 24, 1977		
Sampling site	Total P, %†	Hot H_2O extract. P, %†	Alkaline phosphatase,† Enzyme units* / mg algae	Total P, %	Hot H_2O extract. P, %	Alkaline phosphatase, Enzyme units* / mg algae
Green Bay Campus	.25	.040	25.4	.32	.027	14.6
Bayshore Park	.23	.019	30.0	.24	.023	8.1
Egg Harbor	.17	.023	134.9	.08	.012	190.8
Gill's Rock	.29	.017	52.3	.12	.014	161.7
Bailey's Harbor	.49	.052	660.9	.12	.015	137.1

* One unit of alkaline phosphatase activity equals the amount of enzyme which liberates one millimicromole of nitrophenol/hr, under the prescribed conditions.

† The critical concentration for total P was 0.08%, for hot-water extractable P, 0.013%, and for alkaline phosphatase, 150.

They were not consistently different (Treatments B, C, D, G and M, N, O, P).

Overall the results indicate P is the key nutrient in nuisance *C. glomerata* growths in Green Bay of Lake Michigan. Further sampling probably would result in a similar conclusion for other parts of Lake Michigan. It seems significant that at certain times and at certain sites, *C. glomerata* were collected that were actually P deficient. This indicates not only that P is the key nutrient but also that reduction in P input into Green Bay would reduce nuisance algae growths.

TABLE 7. ASSAYS FOR ADEQUACY OF P AND N SUPPLY FOR Cladophora glomerata GROWTH AT VARIOUS GREEN BAY AND LAKE MICHIGAN SITES SAMPLED SEPTEMBER 18, 1977

Sampling site	Total N, %	Total P, %	Hot H_2O extract. P,* %
Green Bay Campus	2.98	.26	.031
Bayshore Park	2.16	.13	.023
Egg Harbor	2.25	.06	.014
Newport State Park	2.07	.05	.015
Gill's Rock	4.28	.24	.073
Bailey's Harbor	3.29	.14	.035

* Analysis run on dried, ground material rather than fresh material.

While the evidence suggests P is the key factor in _C. glomerata_ growths, the possibility that vitamin B_1 at times might be in that role cannot be eliminated. The evidence for this statement was obtained from the enrichment tests in which yield was reduced as much by omitting vitamin B_1 as by ommitting P. Unfortunately, at this time other assays are not available to confirm this observation. Further studies on the vitamin B_1 nutrition of _C. glomerata_ under field conditions are needed and should be initiated.

Whenever possible, evaluations of relative nutrients in natural waters should be based on several techniques. Several of the bioassays tested, particularly plant analysis, showed promise as simple and reliable tests for nutrient supplies and growth-limiting nutrients for aquatic plant growth.

The general agreement in the three P bioassays was particularly encouraging.

The importance of obtaining relatively clean and healthy Cladophora sp. in order to obtain reliable data from plant analysis and other bioassays should be recognized. In this study, sampling was limited to *C. glomerata* plants that seemed healthy as indicated by a green color. *Cladophora glomerata* is recognized to have heavy growths of epiphytes which could affect inorganic analyses of the algae. As a result, careful attention must be given to removal of as many of the contaminating organisms as possible.

SECTION 4

FURTHER REFINEMENT OF THE PLANT ANALYSIS BIOASSAY FOR NUTRIENT SUPPLIES IN NATURAL WATERS

Plant analysis has been the primary bioassay used on this project to evaluate nutrient supplies and limiting factors for nuisance *Cladophora glomerata* growths. Because plant analysis only recently has been applied to aquatic plants and aquatic nutrient problems (Gerloff, 1973; Gerloff and Fishbeck, 1973), further studies are needed on the reliability of the technique and of factors which might affect the critical concentration values.

The consistency of a specific critical concentration when an organism is grown under widely different environmental conditions is one item of concern. Questions also arise regarding changes in a critical concentration as an organism ages and matures. These changes could be associated with differences in the ratio of protoplasmic to non-protoplasmic constituents arising, for example, from continued deposition of cell wall components or increases in carbohydrate or fat reserves. The results in this section are from several experiments designed to determine the consistency of critical concentrations in *C. glomerata* under varying environmental conditions.

EXPERIMENTAL PROCEDURES

Cladophora glomerata was grown, harvested, and analyzed by procedures described or referred to in Section 3. Deviations from these standard

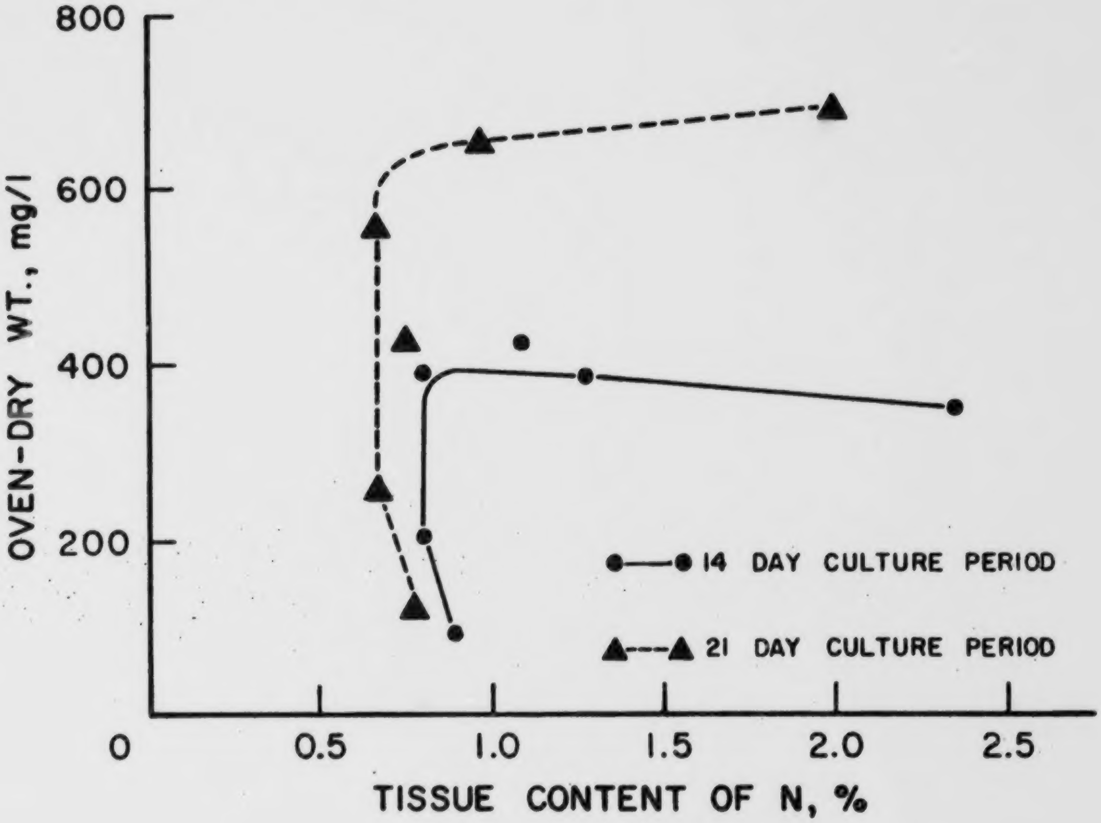

Figure 5. The effect of culture period length on N critical concentration in Cladophora glomerata.

conditions will be mentioned in the presentation of individual experiments.

RESULTS

To determine whether N and P critical concentrations decreased as C. glomerata aged and matured, these values were compared after 14-day and 21-day culture periods. The N data are plotted in Figure 5; the P data, in

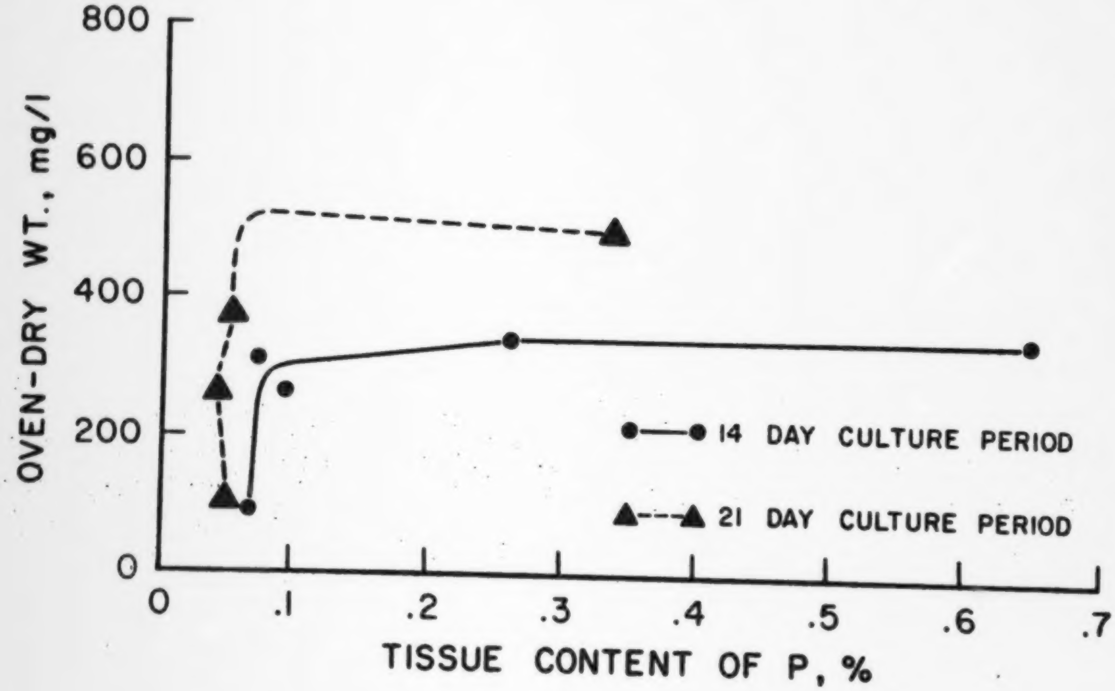

Figure 6. The effect of culture period length on P critical concentration in Cladophora glomerata.

Figure 6. Detailed data and statistical evaluations are in the Appendices (Tables B-5, B-6, A-14, and A-15).

In the N experiment, the maximum yield was approximately 70% greater at 21 days than at 14. Nevertheless, the critical concentration changed only slightly, from approximately 0.9 to 0.8%. The lower value was for

TABLE 8. THE EFFECT OF DIFFERENT PATTERNS OF ADDITION OF A GROWTH-LIMITING AMOUNT OF N TO Cladophora glomerata CULTURES

Treatment*	Ave. algae dry-wt. yield, mg/l+	Ave. N conc. in algae. %
16.4 ppm N, initially	311	3.75
1.6 ppm N, initially	219	.94
0.8 ppm N, initially; at 9 days, .16 ppm N each of 5 days	172	1.10
10 additions of .16 ppm N during 14-day period	160	1.13

* The nutrient medium contained 2.8 ppm P, 9.7 ppm Ca, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace, Na_2CO_3 and Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

+ Average of five replicates in each treatment; 14-day culture period.

the longer culture period. In the P experiment, yield at 21 days was about 60% greater than at 14 days and the critical concentration was lower by approximately 0.01%, that is, it decreased from 0.08 to 0.07%.

The procedure for establishing critical element concentrations in C. glomerata involves "batch culture" of the alga. The total amounts of an element to be made available to the algae in all treatments, from optimal to suboptimal, are added at the start of an experiment. Because of concern that this method of culture might influence critical concentrations, these values were established under several patterns of element addition, as indicated in Tables 8 and 9. There were no significant differences in yields

TABLE 9. THE EFFECT OF DIFFERENT PATTERNS OF ADDITION OF A GROWTH-LIMITING AMOUNT OF P TO *Cladophora glomerata* CULTURES

Treatment*	Ave. algae dry-wt. yield, mg/1[+]	Ave. P conc. in algae, %	SNK[§]
2.8 ppm P, initially	311	.77	a
.07 ppm P, initially	164	.06	b
.035 ppm P, initially; at 9 days, .007 ppm P each of 5 days	152	.06	b
10 additions of .007 ppm P during a 14-day period	179	.06	b

[*] The nutrient medium contained 16.4 ppm N, 9.7 ppm Ca, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace, Na_2CO_3 and Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[†] Average of five replicates in each treatment; 14 day culture period.

[§] Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

and no variations in the critical concentrations when a growth-limiting amount of N or P was made available in one addition or in as many as ten additions during a 14-day culture period.

The experiment reported in Table 10 was designed to determine if growth at a much reduced rate might influence the critical P concentration for *Cladophora glomerata*. A culture temperature of 15C, rather than the

TABLE 10. THE EFFECT OF LOW TEMPERATURE (15 C, ON P CRITICAL CONCENTRATION IN *Cladophora glomerata*

P added to culture soln.,* ppm	Ave. algae dry wt. yield, mg/l†	Ave. P conc. in algae, %	SNK[§]
.07	116	.08	a
.14	173	.08	ab
.28	241	.11	b
.56	270	.15	b
1.12	257	.22	b
2.80	216	.51	ab

* The nutrient medium contained 16.4 ppm N, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 23.4 ppm Ca, 1.12 ppm Fe, 1/5 Johnson's trace, B_7 and C_{13} solutions, vitamin B_1 and B_{12}, and Na_2CO_3 and Na_2SiO_3.

† Average of five replicates in each treatment.

§ Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

usual 23C, was used to reduce the growth rate. As indicated, yields were considerably less than in most *C. glomerata* experiments. Nevertheless, the critical P concentration was in the range of 0.08 to 0.10% and not significantly different than the value established at 23C.

In establishing critical concentrations, all elements are present in

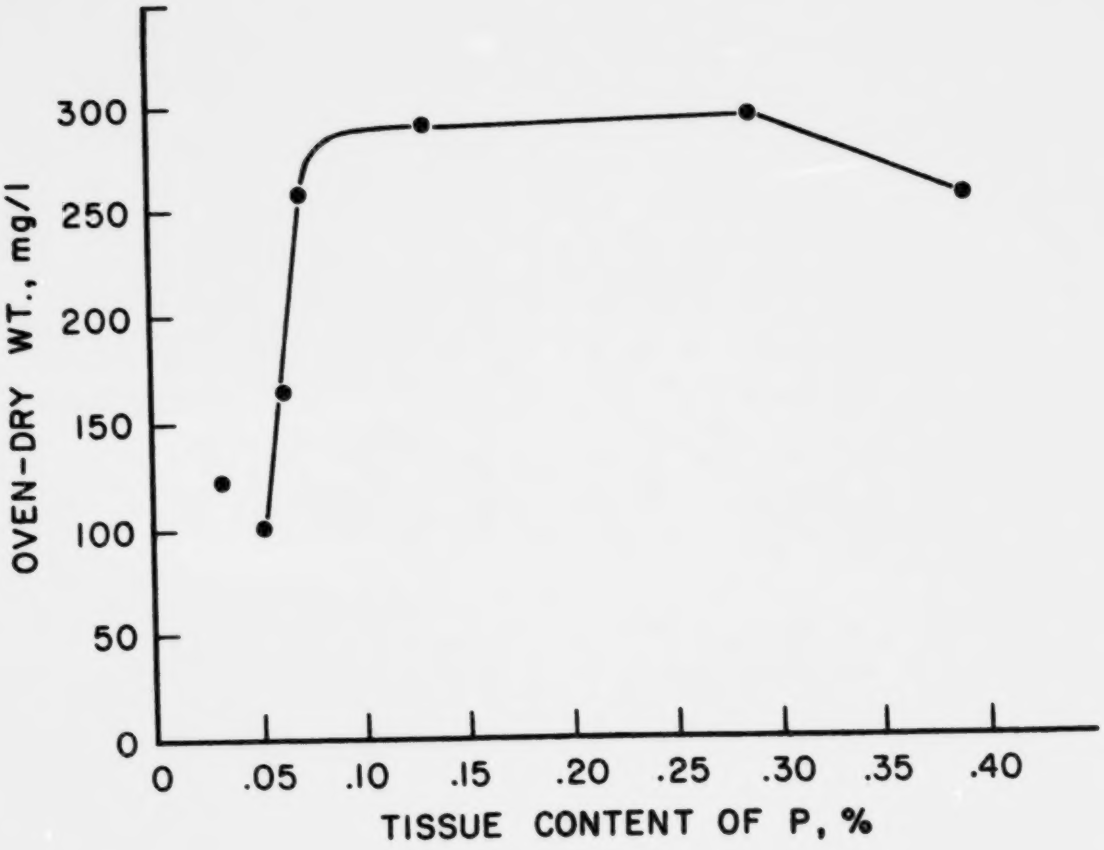

Figure 7. The effect of low K concentration on P critical concentration in Cladophora glomerata.

the culture medium in optimal concentrations except the element for which the critical concentration is being determined. In contrast, under field conditions the supply of more than one element might be growth limiting, or close to growth limiting, at the same time. In the experiment reported in Figure 7, and Appendix Table B-7, the P critical concentration was established when the K concentration in all cultures was constant but less than adequate for optimal growth. Maximum growth was below the average for a 17-day culture period indicating that at the higher P concentrations K supply limited growth. Nevertheless, the P critical concentration was approximately 0.08%, the same as in other tests.

DISCUSSION

The critical N and P concentrations for Cladophora glomerata were found to be relatively unaffected by the age or maturity of the algae and by several environmental factors which affected the rate and amount of algae growth. While additional experiments with C. glomerata, and other organisms as well, are needed, the results obtained contribute significantly to the concept of the critical concentration of an element as a specific value and to the validity of plant analysis as a bioassay for environmental nutrient supplies. With additional confirming data and with careful standardization of sampling procedures, it is anticipated that plant analysis will find the same widespread use for nutrient evaluation in aquatic environments that it currently enjoys in measuring nutrient availability for economic crops in terrestrial environments.

SECTION 5

CONFIRMATION OF UNIQUE NUTRITIONAL FEATURES OF *Cladophora glomerata*

Establishing the exact qualitative and quantitative nutritional requirements of *Cladophora glomerata* in laboratory studies has been a primary aspect of this project. In the early stages, there were indications that *C. glomerata* from Lake Michigan had very high and unusual requirements for S and B. The alga also was shown to require external supplies of vitamin B_1 and very likely vitamin B_{12}, unique requirements for an autotrophic freshwater alga. Because any unusual nutrient requirement could be the basis of measures to control nuisance *Cladophora* sp. growths and because of conflicting reports on the vitamin B_{12} essentiality, further studies were carried out to more closely define requirements for S, B, and vitamin B_{12}.

EXPERIMENTAL PROCEDURES

Cladophora glomerata was cultured, harvested, and chemically analyzed for S and B by procedures detailed or referred to in Section 3.

RESULTS

In the experiment reported in Table 11, *Cladophora glomerata* responded to vitamin B_{12} with approximately a 100% yield increase. There also were statistically significant increases between the first three treatments representing progressively higher B_{12} concentrations. It should be noted that the use of an inoculum which has been through several transfers in

TABLE 11. CONFIRMATION OF QUANTITATIVE RESPONSE OF Cladophora glomerata TO VITAMIN B_{12} IN CULTURE MEDIUM*

Concentration of vitamin B_{12}†, μg/l	Ave. algae dry-wt. yield,§ mg/l
0.00	300 a
0.25	459 b
0.50	622 c
1.0	515 bc
2.0	440 b

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 and C_{13} solutions, vitamin B_1, and Na_2CO_3 and Na_2SiO_3.

† Inoculum grown through three transfers in minus-vitamin B_{12} medium during a seven week period.

§ Average of five replicates in each treatment; yields with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

a medium lacking vitamin B_{12} is essential in demonstrating the requirement. Preparation of vitamin B_{12} was described in an earlier report (Gerloff and Fitzgerald, 1976).

Sulfur

On this project, inorganic nutrient requirements of Cladophora glomerata have been quantitatively expressed primarily as cell critical concentrations, that is the lowest concentrations of elements in algae cells which permit maximum yield. In earlier studies, the critical S concentration in a Cladophora glomerata isolate from Lake Michigan was shown to be 1.50%.

TABLE 12. CRITICAL S CONCENTRATIONS AND THE RANGE OF CONCENTRATIONS IN VARIOUS ALGAE

Algae	Critical S concentration, %	Range in concentration
Lake Huron *Cladophora* sp.	1.70	.90 - 3.46
Lake Erie *Cladophora* sp.	1.20	1.06 - 3.06
Lake Ontario *Cladophora* sp.	1.30	.44 - 2.00
Draparnaldia plumosa	0.15	.02 - .69
Nostoc muscorum	0.13	.11 - .34
Microcystic aeruginosa	0.13	.07 - .21

The critical S concentrations for other algae had not been established; however, for agricultural and horticultural species the range is usually 0.08%-0.25% (Chapman, 1966). To verify the uniqueness of the high *C. glomerata* S requirement, the critical concentration of this element was established for isolates from three additional Great Lakes, for another filamentous green alga (*Draparnaldia plumosa*), and for two species of blue-green algae (*Nostoc muscorum* and *Microcystis aeruginosa*). Data from the individual experiments are reported in Appendix B, Tables B-8 through B-13; the critical concentration established for each organism and the range of S concentration in the algae in each experiment are presented in Table 12.

The critical S concentrations for the three Cladophora sp. isolates were within the range of 1.2 to 1.7%. The correctness of the 1.5% value for the Lake Michigan isolate was verified. The uniqueness of the very high value for C. glomerata was supported by the much lower S critical concentrations for the other algae, 0.13% for the blue-green algae and 0.15% for Draparnaldia plumosa. Both Cladophora glomerata and Draparnaldia plumosa are filamentous green algae, yet the critical S concentration is 7x greater for C. glomerata.

In an early phase of this project, samples of Cladophora sp. were collected from a number of sites on four of the Great Lakes. Where sufficient plant material remained these samples were analyzed for S. As shown in Table 13, in spite of the very high requirement, there was little indication that S supply became a limiting factor in the growth of Cladophora sp. In one sample, from inside the Milwaukee breakwater on 7-8-74, the S concentration was only 1.5%; in most samples, the concentration was 2.0% or more.

Boron

In the initial phase of this project, the critical B concentration for Cladophora glomerata from Lake Michigan was established as 110 ppm which is far above the 5-25 ppm critical concentrations for most plant species and for other algae (Chapman, 1966; Gerloff, 1968). The data in Table 14 are from an effort to establish the critical B concentration in a C. glomerata isolate from Lake Erie. There were statistically significant yield decreases at the lowest levels of B in the culture medium. However, even in the B deficient algae the B concentration was in excess of 150 ppm which not only confirms the earlier result but suggests the 110 ppm critical concentration was conservatively low.

TABLE 13. SULFUR ANALYSIS OF *Cladophora* sp. COLLECTED FROM THE GREAT LAKES

Lake sampled	Sampling site	Date sampled	S conc., %
Michigan	Holland	10-18-73	2.3
Michigan	Manistique	7-29-74	>3.0
Michigan	Milwaukee, N. Pt. Park	9- 6-73	3.0
Michigan	Milwaukee, N. Pt. Park	10-14-73	1.8
Michigan	Milwaukee, N. Pt. Park	7- 8-74	2.2
Michigan	Milwaukee breakwater, shore, inside	6-17-74	2.5
Michigan	Milwaukee breakwater, shore, outside	6-17-74	>1.9
Michigan	Milwaukee breakwater, shore, inside	7- 8-74	1.5
Michigan	Milwaukee breakwater, shore, outside	7- 8-74	2.5
Michigan	Milwaukee breakwater, 0.5 mile, inside	7- 8-74	2.0
Michigan	Milwaukee breakwater, 0.5 mile, outside	7- 8-74	2.3
Michigan	Milwaukee, Pier 2, inside	7-16-74	1.9
Michigan	Milwaukee, Pier 2, outside	7-16-74	>3.0
Michigan	Milwaukee, Pier 4, inside	7-16-74	2.0
Michigan	Door County, Egg Harbor	9-11-74	1.9
Michigan	Door County, Gill's Rock	9-11-74	3.4
Michigan	Menominee	9-12-74	>2.9
Erie	Western, Port Clinton, Ohio	6-20-74	1.9
Erie	Eastern, Geneva, Ohio	6-20-74	2.5
Erie	Eastern, Ft. Erie, Ontario	8-11-74	2.9
Erie	Eastern, Pt. Colburne, Ontario	8-12-74	2.6
Ontario	Olcott, New York	6-20-74	1.9
Huron	Pt. Sanilac, Michigan	10-19-73	2.3

TABLE 14. YIELD AND B CONCENTRATION OF Cladophora glomerata ISOLATED FROM LAKE ERIE GROWN IN CULTURE MEDI DIFFERING IN B CONCENTRATION

B added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	Ave. B conc. in algae, ppm	SNK§
0.00	287	178	a
0.00054	343	198	ab
0.0011	423	161	bc
0.0054	458	238	c
0.054	520	249	c
0.27	525	264	c

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements (-B), Na_2CO_3, Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}. Culture flasks were low boron, alkali-resistant glassware.

† Average of six replicates in each treatment except for the two highest treatments which had five replicates each.

§ Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

DISCUSSION

The unique requirement of Cladophora glomerata for an external supply of vitamin B_{12} and its unusually high requirements for S and B have been verified. Nevertheless, there is no indication from the results in this section, and from Section 3, that these nutrients play a critical role in the field nutrition of Cladophora glomerata. The key nutrient instead is the element P for which the critical concentration is relatively low (0.08%) compared to values for other algae and macrophytes (Gerloff, 1969). Natural and pollution sources of S, B, and vitamin B_{12} apparently are providing adequate amounts of these nutrients to the parts of the Great Lakes sampled.

Even though the very high S and B requirements seem to be of little practical importance, they should be of considerable basic physiological interest. The distribution in the cells and the biochemical utilization of S which accounts for the 7x greater S requirement of _C. glomerata_ compared to _Draparnaldia plumosa_ deserves further study. The high B requirement of _C. glomerata_ is particularly of interest in view of the complete lack of a requirement in some algae and fungi (Gerloff, 1968). The physiological basis for these variations undoubtedly will remain unsettled until the functions of B in plant structure and/or metabolism are established. Because of its unique requirement, _Cladophora glomerata_ should be a useful organism in such studies.

TABLE B-13. YIELD AND S CONCENTRATION IN THE BLUE-GREEN ALGA *Microcystis aeruginosa* GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

S added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	Ave. S conc. in algae, %
0.12	54	.07
0.24	61	.08
0.49	166	.10
0.97	203	.15
1.94	207	.16
4.85	192	.16
9.70	207	.19
19.4	177	.21

* The nutrient medium contained 82 ppm N, 18 ppm K, 7 ppm P, 7.5 ppm Mg, 9.7 ppm Ca, 1.12 ppm Fe, 1/5 Johnson's trace elements. and Na_2CO_3 and Na_2SiO_3.

† Average of four replicates in each treatment.

SECTION 6

GROWTH OF *Cladophora glomerata* AT ELEMENT CONCENTRATIONS APPROXIMATING LAKE CONCENTRATIONS

Aquatic plants usually are cultured in the laboratory in nutrient media containing much higher concentrations of the essential elements than are present in lakes and streams. This is necessary to provide the total amounts of the elements necessary for high yields in the small volumes of nutrient solution provided. As a result, the responses of aquatic organisms to element concentrations determined in the laboratory usually cannot be directly related to field conditions.

Responses of *Cladophora glomerata* to the very low concentrations of N, P, and other elements characteristic of natural waters can be measured in various ways. This includes the use of very large volumes of culture medium, continuous-flow cultures, the frequent addition of an element to a culture to maintain a very low concentration, and the uptake of a radioactive tracer at low concentrations over relatively short time periods. Our approach was to progressively lower the concentration of one element, while making an adequate total amount available, until a concentration was attained at which the algae could not absorb the element rapidly enough to produce maximum growth. This point was evaluated by comparisons with growth in cultures provided with a normal supply of the element under study. The relative aggressiveness of *Cladophora* in the uptake of specific elements could be evaluated by comparisons of minimum solution concentrations for maximum growth of that species with comparable concentrations for other nuisance algae and macrophytes.

EXPERIMENTAL PROCEDURES

Cladophora glomerata was confined to a culture vessel of relatively small volume (2 l) and large volumes of solution were made available to the alga by draining away and replacing the culture medium at frequent intervals. Because of the large volumes of nutrient solution required, only two treatments, which were replicated, could be included in each experiment. In every experiment, one treatment always was the control solution consisting of *Cladophora* medium (Gerloff and Fitzgerald, 1976) at one-quarter strength; the other treatment was similar except the element under study was provided at a very low concentration. Experiments were repeated, with a progressively lower concentration of the limiting element made available in each successive experiment, until a concentration was established at which rate of absorption was inadequate to provide yields equal to those in the control solution.

For significant differences in dry weight to be detected in the solution replacement cultures, growth periods of 10 to 14 days were necessary, yielding tissue dry weights of 75 to 100 mg per replicate. The lowest solution concentration that could be tested was a function of the critical concentration (%) for the controlled nutrient multiplied by the increment of total growth (mg) produced in the last day of the culture period divided by the largest culture medium volume (liters) it was practical to make available to the algae in 24 hours. At least twice this "minimum daily requirement" of the controlled element was made available to the *Cladophora* even at the lowest concentration tested.

Two additional procedures were tested for establishing minimum nutrient concentrations for optimum Cladophora glomerata growth. One approach was to monitor the depletion of P using ^{32}P as a radioactive tracer. Whenever the activity of a culture fell below 50% of the activity of a sterile uninoculated standard, sufficient P was added to the culture to equal the activity in the standard. In this way, the P concentration was controlled between 0.5 and 1.5x the concentration of the standard.

Radioactivity counts were made both on unfiltered and millipore-filtered aliquots from the cultures. This was to determine if any separated particles or microorganisms were sequestering a substantial pool of the P. This was a critical point, because P tied up in particulate debris might not be available to the C. glomerata yet would be indicated as available P by radioactivity counts. No substantial pool of organic P developed in the medium. However, because of problems from algae contaminants introduced by the repeated opening of the culture vessels, this technique was abandoned in favor of frequent replacement of the culture solution.

Another approach attempted was to reduce the algae tissue cultured to a microscopic quantity and thus avoid the need for nutrient solution replacement altogether. Quarter-strength medium was placed in petri dishes into which C. glomerata filaments of 5 to 8 cells each were placed. Growth of the filaments was to be followed by microscopic examination to determine increase in cell members and filament volume.

The problems of maintaining sterility and concentration were obviated by this approach. However, two problems with the system could not be solved. First, the variation in the vigor of randomly selected filaments was very large, and second a practical and objective procedure to determine the growth of individual filaments could not be established. Counting cells, counting branch points on the filaments, and measuring the length of the filaments were attempted, but the considerable variation in the size of *C. glomerata* cells, the degree of branching, and the width of filaments made all of these indices unsatisfactory.

RESULTS

The data in Table 15 show the results from three experiments in which *Cladophora glomerata* was grown at P concentrations approximating those in lakes and streams. In Experiment 1, the lower P concentration was 0.028 ppm. Yield was not significantly different than in the control culture containing 1.4 ppm P. In experiments 2 and 3, the P concentration was reduced to 0.014 ppm, and in both cases *C. glomerata* growth was reduced significantly. In Experiment 2 the dry weight yield was 51% of the control; in Experiment 3, 80%.

It was anticipated that P concentrations in *C. glomerata* grown at 0.014 ppm P would be near or below the critical concentration of 0.08%. This was true in Experiment 3 in which the P concentration was 0.09%. However, in Experiment 2 the *C. glomerata* P content was surprisingly high at 0.16%.

Only 26 and 36% of the P added in the 0.014 ppm solution was absorbed in Treatments 2 and 3 respectively. This supports the view that, as desired, the pattern of solution replacement employed made much more P available to the algae than could be absorbed.

Cladophora glomerata yield was significantly reduced when the K concentration in the culture medium was reduced from 9.0 to 0.18 ppm. Of perhaps greater interest was the nearly complete failure of *C. glomerata*

TABLE 15. RESPONSE OF *Cladophora glomerata* TO LOW CONCENTRATIONS OF ESSENTIAL ELEMENTS MADE AVAILABLE IN NUTRIENT SOLUTION REPLACEMENT CULTURES

				Cladophora yield		Element in *Cladophora*			
Element varied	Element conc., ppm	Soln. volume, l	Element added, mg	Dry weight, mg	Relative, %	Conc., %	Weight, mg	Added element absorbed,* %	t-test
P	1.4	14.2	19.90	73.9	100	0.69	0.51	3	N.S.
	0.028	14.2	0.40	71.7	97	0.13	0.09	23	
P	1.4	22.2	31.08	99.5	100	0.75	0.75	2	< 0.05
	0.014	22.2	0.31	50.6	51	0.16	0.08	26	
P	1.4	14.2	19.88	100.1	100	0.87	0.87	4	< 0.02
	0.014	14.2	0.20	79.8	80	0.09	0.07	36	
K	9.0	17.2	154.80	147.7	100	4.92	7.27	5	< 0.10
	0.18	17.2	3.10	101.3	69	2.47	2.51	81	
Ca	4.77	5.2	24.69	30.2	100	--	--	--	< 0.001
	0.24	5.2	1.24	4.3	14	--	--	--	

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* Includes amount of element added in inoculum.

to grow when the Ca concentration was lowered to 0.24 ppm. The amount of plant material produced was insufficient for Ca analyses.

Two tests were made on *C. glomerata* response to low N concentrations. The data were not sufficiently conclusive to include in this report.

DISCUSSION

As suggested in an earlier report (Gerloff, 1975), the data presented in this section are of considerable interest in relation to the interpretation of chemical analyses of water samples. A water concentration considered limiting for nuisance plant growths may be limiting not only because the amount of an element required for a specific amount of growth is inadequate, but also because the concentration is so low that the rate of absorption cannot keep pace with needs for luxuriant growths. In other words, it cannot be assumed that each unit of a nutrient is equally available to organisms and equally effective in promoting growth over the range of concentrations present in natural waters.

The minimal solution concentrations at which maximum growth can be maintained undoubtedly varies for different aquatic species, but so far only limited data are available for such comparisons. One example is the *C. glomerata* results and data on the macrophyte *Elodea occidentalis* (Gerloff, 1975). At 0.03 ppm P in solution replacement cultures, *Elodea* growth was significantly below the control; *C. glomerata* yield was not reduced at 0,028 ppm but was at 0.014 ppm. *Cladophora glomerata*, therefore, seems more aggressive than *Elodea* in P uptake and utilization.

Cladophora glomerata is most abundant in hard water lakes in which Ca and Mg concentrations are relatively high. This correlates with the complete inability of *C. glomerata* to grow with only 0.24 ppm Ca in the nutrient solution. Again comparisons with other organisms become of interest. In the study mentioned above (Gerloff, 1975), the filamentous green alga Draparnaldia plumosa made optimal growth in a solution containing 0.80 ppm Ca while Elodea occidentalis made almost no growth in the same solution. Information obtained with solution replacement cultures, or another similar technique, might contribute significantly to an interpretation of aquatic plant distribution in hard water and soft water lakes.

Solution replacement cultures as described require much time and effort. However, they have the advantage of indicating responses to element concentrations that are reflected in actual plant growth. This may not always be true when potential for element utilization is measured with radioactive isotope uptake over short intervals. Techniques based on plant growth also minimize another complication of isotope uptake studies, namely that uptake of an element is greatly influenced by the degree of deficiency of that element in the plants used as experimental material.

SECTION 7

AN ADDITIONAL ESSENTIAL ELEMENT FOR *Cladophora glomerata*

In the early stages of this project, evidence was obtained that *Cladophora glomerata* requires at least one inorganic nutrient currently unrecognized as essential for plant growth. This was demonstrated through *C. glomerata* yield response when B_7 and C_{13} solutions (mixtures of elements of suspected essentiality) were added to *C. glomerata* cultures. It was anticipated that systematic omission of elements from the B_7 and C_{13} solutions would quickly establish which element was responsible for the beneficial effects of the supplemental mixtures. This has been the approach in extending the list of essential elements for other species. As indicated in the following paragraphs, in spite of a number of experiments, the results have not been as anticipated and the issue has not been settled.

EXPERIMENTAL PROCEDURES

Establishing the essentiality of additional inorganic nutrients usually requires special purification procedures to remove element contamination from the reagent salts from which the synthetic culture medium is prepared. The definite response when B_7 and C_{13} were omitted from the culture medium made these lengthy purification procedures seem unnecessary on this project. Careful attention was given to contamination from other sources, for example in thorough cleansing of all glassware in acid, in the

TABLE 16. RESPONSE OF Cladophora glomerata TO THE ADDITION OF POTENTIALLY ESSENTIAL MICRONUTRIENTS

Treatment	Ave. algae dry-wt. yield,* mg/l	
	Exp. I	Exp. II
Cladophora medium+	59a	113a
Cladophora medium, plus B_7, C_{13}§	536b	291b
Cladophora medium, plus B_7	--	111a
Cladophora medium, plus C_{13}	--	246b

* Average of ten replicates in each treatment in Experiment I, and four replicates in each treatment in Experiment II; yields with a common letter are not significantly different using Student-Newman-Keuls multiple range test.

+ The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, Na_2CO_3, Na_2SiO_3, and vitamins B_1 and B_{12}.

§ The B_7 trace element mixture contained 0.001 ppm of each of the following: V, Cr, Ni, Co, W, Ti, and Sn. The C_{13} trace element mixture contained 0.0005 ppm of each of the following: Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, and Be.

use of water double-distilled from glass in preparing culture media, and in carrying C. glomerata to be used as innoculum through several transfers in a minus B_7 C_{13} medium.

RESULTS

The data in Table 16 show the response of C. glomerata to the elements in B_7 and C_{13} solutions in two experiments. Yield increase was statistically significant in both experiments. In Experiment II, omitting the C_{13} solution decreased yield from 291 to 111 mg/liter; omitting the B_7 solution was

TABLE 17. RESPONSE OF *Cladophora glomerata* TO THE ADDITION OF B_7 MICRONUTRIENTS

Treatment	Ave. algae dry-wt. yield,* mg/l
Cladophora medium, plus B_7 †	529
Cladophora medium, minus B_7	582
Cladophora medium, minus V	588
Cladophora medium, minus Cr	529
Cladophora medium, minus Ni	633
Cladophora medium, minus W	525
Cladophora medium, minus Ti	581
Cladophora medium, minus Sn	515
Cladophora medium, minus Co	498

* Average of five replicates in each treatment; yields are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, C_{13} solution, Na_2CO_3, Na_2SiO_3, and vitamins B_1 and B_{12}. The B_7 trace element mixture contained 0.001 ppm of each of the following: V, Cr, Ni, Co, W, Ti, and Sn.

without significant effect. The element, or elements, responsible for the yield increase seem to be among the C_{13} components.

Tables 17 and 18 present data from experiments designed to establish the specific B_7, C_{13} components that stimulated growth. As anticipated, there was no significant yield reduction when the B_7 solution or each element of the B_7 solution was omitted in individual treatments (Table 17).

TABLE 18. RESPONSE OF Cladophora glomerata TO THE ADDITION OF C_{13} MICRONUTRIENTS

Treatment	Ave. algae dry-wt. yield,* mg/l	
	Exp. I	Exp. II
Cladophora medium, plus C_{13}†	427	408
Cladophora medium, minus C_{13}	327	289
Cladophora medium, minus C_{13} "7"§	238	304
Cladophora medium, minus As	407	334
Cladophora medium, minus Cd	447	360
Cladophora medium, minus Hg	416	384
Cladophora medium, minus Pb	454	366
Cladophora medium, minus F	520	346
Cladophora medium, minus Se	434	341
Cladophora medium, minus Be	427	375

* Average of five replicates in each treatment; yields are not significantly different within each experiment at the 5% level using Student-Newman-Keuls multiple range test.

† The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 solution, Na_2CO_3, Na_2SiO_3, and vitamins B_1 and B_{12}. The C_{13} solution contained 0.0005 ppm of each of the following: Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, and Be.

§ The C_{13} "7" solution contained the elements individually tested in the experiment: As, Cd, Hg, Pb, F, Se, and Be. The remaining six elements (Al, Sr, Li, Rb, Br, and I) were added.

There was reason to believe that the C_{13} response in Table 16 was due to one of the following C_{13} elements: arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), fluorine (F), selenium (Se), or beryllium (Be). However, omitting each of those elements from the culture medium was without effect on yields in both experiments reported in Table 18.

Poor agreement among replicates within treatments seemed to be a major reason for the inconsistencies in the B_7, C_{13} experiments. The poor replication in turn was due to uneven initiation of growth from the vegetative filaments used as inoculum. To provide more uniform yields, an effort was made to induce *C. glomerata* to produce zoospores which in turn would serve as inoculum. It was established that zoospore release could be accomplished when a two-week old *C. glomerata* culture which had been under conditions of high continuous light (800 ft-c) and high temperature (25C) for three to four days was transferred to fresh culture medium and placed under short days (8 hours), low light (200 ft-c or less), and low temperature (21C). The cultures were watched closely for zoospore release at approximately three days. When this occurred, the zoospores were concentrated by millipore filtration and small volumes were used as innoculum. The zoospores immediately began to attach to the sides of the flasks.

Because of termination of the project, only one experiment with zoospore innoculum has been possible (Table 19). Overall the results confirm the data from Table 16. Omitting the C_{13} solution, or the C_{13} "7" components of the C_{13} solution, decreased yield from 741 to only 12 to 14 mg/liter. However, completely unexpected results were obtained when the C_{13} "7" components were individually omitted from the culture solution. No element had a significant effect on *C. glomerata* yield. Although detailed data are not given, replication was improved when zoospores rather than vegetative filaments were used as innoculum.

TABLE 19. RESPONSE OF Cladophora glomerata TO THE ADDITION OF C_{13} MICRONUTRIENTS USING ZOOSPORES AS INOCULUM

Treatment	Ave. algae dry-wt. yield,*mg/l
Cladophora medium, plus C_{13}†	741
Cladophora medium, minus C_{13}	12
Cladophora medium, minus C_{13} "7"§	14
Cladophora medium, minus As	563
Cladophora medium, minus Cd	564
Cladophora medium, minus Hg	776
Cladophora medium, minus Pb	783
Cladophora medium, minus F	778
Cladophora medium, minus Se	626
Cladophora medium, minus Be	736

* Average of five replicates in each treatment; 28-day culture period.

† The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 solution, Na_2CO_3, Na_2SiO_3, and vitamins B_1 and B_{12}. The C_{13} solution contained 0.0005 ppm of each of the following: Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, and Be.

§ The C_{13} "7" solution contained the elements individually tested in the experiment: As, Cd, Hg, Pb, F, Se, and Be. The remaining six elements (Al, Sr, Li, Rb, Br, and I) were added.

DISCUSSION

The inability to translate the strong response to the C_{13} solution into a requirement for an additional essential element has been frustrating and unlike experience in the studies that led to the essentiality of other elements, for example Mo (Arnon and Stout, 1939) and Cl (Broyer et al, 1954) for all plants and Co for some plants (Ahmed and Evans, 1961; Delwiche, et al, 1961). One possible explanation for failure in this study is that the unrecognized essential element is an impurity in several of the C_{13} "7" salts. The presence of any one of those salts provides enough of the unrecognized essential element to meet the needs of Cladophora glomerata. This seems doubtful because of the extremely low concentration in which the C_{13} components were added (0.0005 ppm of each element). Another possibility is that an additional element is essential for C glomerata but another element can substitute for it. Such substitutions have been established among other elements, for example between K and Na and Sr and Ca (Walker, 1956; Brian, 1967).

Considerable additional work probably will be necessary to clarify the situation discussed. The improved replication within treatments in the last experiment suggests that for future experiments zoospores should be used as innoculum. Because of the very small quantities that would be required, it seems unlikely that the unknown essential element would be of practical importance in the nutrition and ecology of Cladophora in the Great Lakes. Nevertheless, the discovery of a new essential element for even a single species is an exciting scientific event. For that reason, further studies to clarify the situation discussed seem worthwhile.

SECTION 8

HEAVY METAL TOXICITY TO *Cladophora glomerata*

Because of increased interest in the effects of heavy metals likely to be introduced into aquatic environments on plants and animals, tests were made on the responses of *Cladophora glomerata* to several common heavy metal pollutants often toxic at relatively low concentrations. This was not a part of the original project proposal. However, the experience in culturing *Cladophora glomerata* on this project made expansion into toxicity studies relatively simple and worthwhile.

Organism response to toxic agents can be expressed in various ways. A common expression is the environmental concentration which results in a 50% depression in organism numbers or growth in a standardized laboratory test. A disadvantage of this approach is that the volume of a solution, as well as the concentration of a toxic agent, determines the total amount of a toxic agent to which an organism is exposed. For this reason, the Principal Investigator prefers expressing toxicity of metals as minimum concentrations in organisms, tissues, and cells which are responsible for significant decreases in yield.

EXPERIMENTAL PROCEDURES

Cladophora glomerata was grown in the medium and under the conditions developed as standard culture technique on this project and described in earlier sections. Each culture involved 200 ml of medium in a 500 ml

TABLE 20. RESPONSE OF Cladophora glomerata TO VARIOUS CONCENTRATIONS OF COPPER

Cu added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	SNK§
0.03	726	a
0.15	800	a
0.18	773	a
0.21	655	ab
0.24	530	b
0.30	252	c

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements (-Cu), Na_2CO_3, Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

† Average of five replicates in each treatment.

§ Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

Erlenmeyer flask. All heavy metals were aseptically added to the cultures after autoclaving. The inoculum was a 1-2 mg oven-dry weight segment of vegetative filaments from a vigorous culture.

RESULTS

The yield responses to various concentrations of copper (Cu), manganese (Mn), cadmium (Cd), lead (Pb), and zinc (Zn) are presented in Tables 20 through 24. Copper significantly reduced yields at solution concentrations of 0.24 ppm and higher. Cladophora glomerata tolerated 1.35 ppm Mn but was affected adversely by 2.16 ppm of that element. Cadmium was not harmful

TABLE 21. RESPONSE OF Cladophora glomerata TO VARIOUS CONCENTRATIONS OF MANGANESE

Mn added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l
0.00	356
0.27	698
1.35	606
2.16	342
2.43	296
2.70	188

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements (-Mn), Na_2CO_3, Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

† Average of five replicates in each treatment; a statistical analysis of the data can be found in Appendix A (Table A-24).

at 1.0 ppm; 2.5 ppm of the element, however, completely killed C. glomerata. Lead was relatively non-toxic with no reduction in yield at 3.0 ppm, the highest concentration tested. Zinc was surprisingly toxic with growth nearly stopped at a solution concentration of 0,65 ppm.

DISCUSSION

Time was not available in the final stages of this project for analyses of Cladophora glomerata and expressions of toxicity as cell concentrations of the heavy metals tested. The samples have been stored for possible analyses in the future. At this time, only general observations on toxicities to C. glomerata in relation to solution concentrations and by

TABLE 22. RESPONSE OF Cladophora glomerata TO VARIOUS CONCENTRATIONS OF CADMIUM

Cd added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l
0.0	396
0.1	399
0.4	290
0.6	303
1.0	286
2.5	0

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 and C_{13} solutions, Na_2CO_3, Na_2SiO_3, and vitamins B_1 and B_{12}.

† Average of five replicates in each treatment; yields of treatments 0.0 through 1.0 ppm Cd are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

comparison with toxicities to other organisms are possible.

Copper salts are among the most widely used algicides at copper ion concentrations of 0.2 to 0.5 ppm. This correlates with the yield reduction to about one-third of maximum in a solution containing only 0.3 ppm Cu.

Zinc and manganese are both essential elements for plants and elements for which the range between toxicity and essentiality often is relatively narrow. Probably because Mn toxicity occurs in the field in very acid soils, much information is available on the response of various plant species to Mn. Some legumes are affected by only 1.0 ppm Mn in the culture soltuion; some cereals will tolerate much higher concentrations of the

TABLE 23. RESPONSE OF *Cladophora glomerata* TO VARIOUS CONCENTRATIONS OF LEAD

Pb added to culture soln.,[*] ppm	Ave. algae dry-wt. yield,[†] mg/l	SNK[§]
0.0	213	ab
0.1	99	a
0.6	287	ab
1.0	386	b
2.0	250	ab
3.0	252	ab

[*] The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, Na_2CO_3, Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[†] Average of five replicates in each treatment.

[§] Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

element (Hewitt, 1966). On the basis of higher plant data, *Cladophora glomerata* seems moderately susceptible to Mn toxicity. In contrast, *C. glomerata* appears quite susceptible to Zn toxicity. The Zn concentrations employed in higher plant cultures generally are in the range of 0.1 to 0.5 ppm. *Cladophora glomerata* growth was almost completely stopped by 0.65 ppm of Zn.

Cladophora glomerata seemed quite tolerant of Cd, one of the two non-essential heavy metals tested. Some testing of the response of economic plants to Cd has been carried out because of concern over possible problems resulting from land disposal of sewage sludge. Growth of beets, beans, and

TABLE 24. RESPONSE OF Cladophora glomerata TO VARIOUS CONCENTRATIONS OF ZINC

Zn added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l
0.00	637
0.13	486
0.65	23
0.91	20

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm Fe, 1/5 Johnson's trace elements (-Zn), Na_2CO_3, Na_2SiO_3, B_7 and C_{19} solutions, and vitamins B_1 and B_{12}.

† Five replicates in each treatment.

turnips was reduced by 50% at a Cd solution concentration of 2 ppm; similar growth reduction in corn resulted at 1 ppm Cd and in tomatoes and barley at 5 ppm. Cabbage was the most tolerant plant tested with 9 ppm Cd required for a 50% yield reduction (Page, et al, 1972). Cladophora glomerata tolerance of Pb cannot be evaluated until additional experiments are carried out to establish concentrations which decrease growth,

On the basis of experience with higher plants, and particularly crop plants, it can be anticipated that aquatic species will be highly selective in their accumulation of heavy metals and in their responses to high concentrations of the metals. It seems important to begin accumulating basic information of this nature for the most prevalent aquatic plants in the Great Lakes. With the input of large amounts of specific heavy metals in local areas, those metals may become critical in the ecology of the polluted water. Knowledge of the responses of aquatic species to the metals could be useful in predicting changes in the dominant species as a result of heavy metal pollution.

SECTION 9

THE CULTURE AND NUTRITION OF THE DIATOM *Nitzschia palea*

Explanations of the abundance of *Cladophora glomerata* in certain areas and under specific conditions probably would be facilitated if detailed information were available not only on the nutritional requirements and responses of *C. glomerata* but also on the primary organisms with which it occurs and competes. Diatoms were selected for additional nutritional studies because they are the dominant organisms in all the Great Lakes (Schelske and Stoermer, 1971).

This section reports the isolation from Lake Michigan *C. glomerata* filaments of the diatom *Nitzschia palea*, the laboratory culture of the organism in a synthetic medium, and its general nutrient requirements.

EXPERIMENTAL PROCEDURES

Diatoms were collected from Green Bay near the entrance to Sturgeon Bay by wiping filaments of *Cladophora glomerata* across solidified medium which contained 25% lake water and 1.5% agar in petri dishes. The medium was a slightly modified Hughes, Gorham, Zehnder (1958) solution. The petri dishes were transferred to the lab the same day and placed in a culture room.

Within a week, algal colonies appeared on the agar. Diatoms were transferred aseptically with a flamed wire loop to liquid culture medium to which 25% by volume lake water had been added. The organisms were allowed to grow for approximately two weeks before restreaking onto agar. This not only facilitated isolation but also selected organisms which grow well in liquid media. This procedure was repeated until unialgal cultures were obtained as determined by microscopic examination. One isolate which grew faster and more abundantly than the others was selected for detailed study. Efforts were made to obtain bacteria-free cultures by several procedures. Although bacterial numbers could be reduced by treatment with an antibiotic mixture, axenic cultures were not obtained.

Cleaned diatom frustules were obtained by the standard procedure given by Patrick and Reimer (1966). Permanent slides were obtained by using pleurax, a synthetic mounting medium developed by Hanna (1949) which is commonly used in diatom identification because of its high refractive index.

Permanent slides and micrographs were sent to Dr. Reimer at The Academy of Natural Sciences in Philadelphia who identified the organism as _Nitzschia palea_ var. _tropica_ Hust. This assumes that no changes in wall structure had taken place during the time the organism was in culture.

Apparatus and Culture Conditions

Unless otherwise stated, cultures were maintained at 23 ± 1C under continuous fluorescent light of 350-500 ft-c derived from T-12 cool-white bulbs.

The diatoms were grown in 500 ml Erlenmeyer flasks containing 200 ml of liquid culture medium. Humidified and filtered air was provided by incorporating a double-distilled water trap and a polyester fiberfill filter into the aeration system. The treated air was then gently bubbled through the culture medium.

Routine Culture Procedure

All glassware coming into contact with the culture media was washed, rinsed in distilled water, soaked in $4\underline{N}$ HNO_3, and thoroughly rinsed in distilled and double-distilled water (second distillation in a glass still). Media were prepared in large batches, poured into the 500 ml Erlenmeyer culture flasks, and sterilized by autoclaving at 15 psi for 15 min. Only water which had been double-distilled was used in media preparation.

The diatoms generally were harvested between the period of most rapid growth and senescence, the exact timing depending upon the type of experiment. Harvest was by centrifugation at 2,000 RPM for one minute at 0C in tared, plastic 50 ml centrifuge tubes. The tubes were then oven-dried, cooled, reweighed, and the dry weight was recorded for cells from each flask. Statistical analyses were run on the results of the experiments using Duncan's multiple range test.

RESULTS

Media Modification Experiments

Figure 8 presents the combined results of the initial experiments which were most effective in increasing *Nitzschia palea* yield. This was accomplished by increasing the $Na_2SiO_3 \cdot 9H_2O$ concentration in the medium (5.7 to 46 ppm Si) and substituting $Ca(NO_3)_2$ for $NaNO_3$. The combined modifications resulted in a yield of approximately 800 mg/l in contrast to 80 mg/l in the original medium.

Although these modifications produced an adequate yield, the resulting medium was unacceptable for *N. palea* nutritional studies. The high concentrations of Na_2SiO_3 $9H_2O$ and $Ca(NO_3)_3$ produced a heavy precipitate which

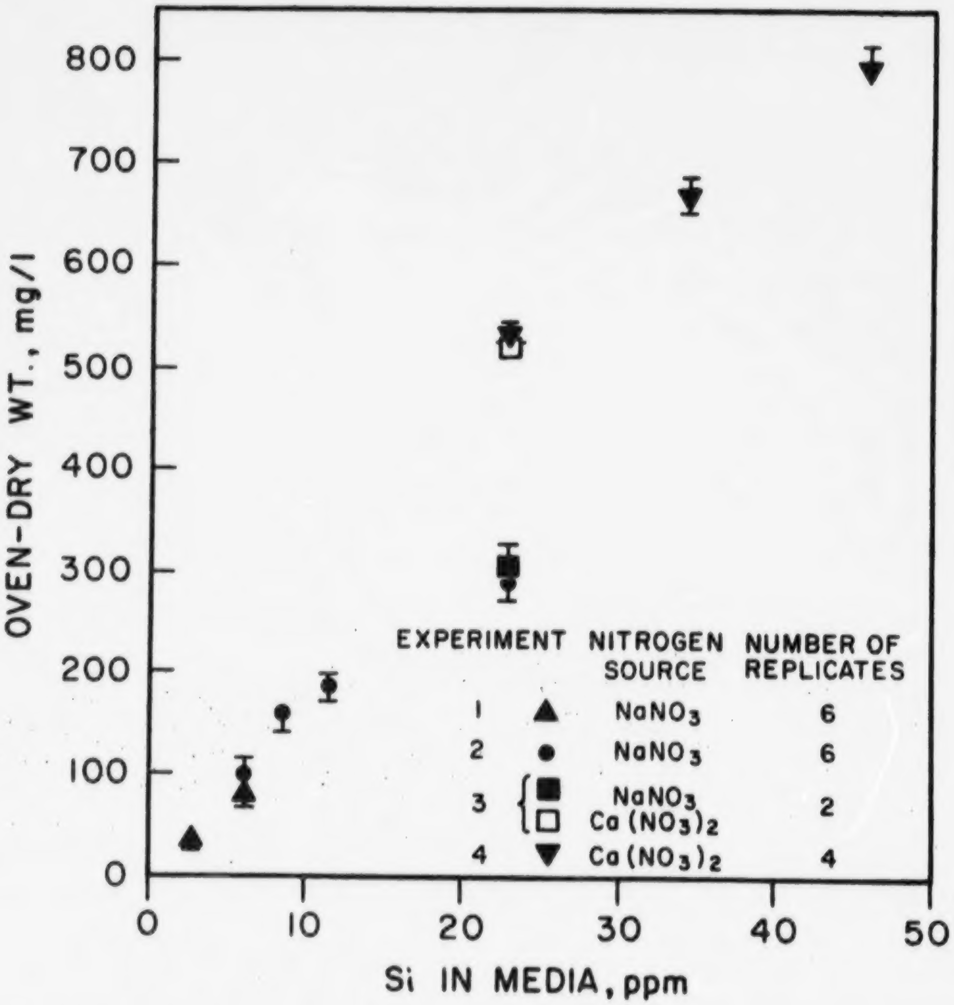

Figure 8. The effect on *Nitzschia palea* yield of increasing the media Si concentration and substituting $Ca(NO_3)_2$ for $NaNO_3$.

formed immediately upon addition of the salts. It was difficult to determine the quantity of precipitate remaining at the end of the culture period, thus making it impossible to obtain accurate diatom yields.

The precipitate problem finally was resolved by using silicic acid as a silicon source. This chemical, although sparingly soluble in water at a pH near neutral, dissolves upon heating in a strong basic solution. Experiments showed that at low silicon concentrations, the silicic acid remained in solution upon readjustment of the pH to neutral with HCl. Because a gel formed at high silicon concentrations, it was most convenient to prepare a silicon solution of neutral pH in large quantities instead of smaller amounts of a highly concentrated stock solution. Specific details on preparation of the recommended silicon solution (containing approximately 55 ppm Si) are given in Table 25.

Sodium silicate and silicic acid solutions are compared as a silicon source for _N. palea_ in Table 26. The recommended silicic acid solution not only gave a higher yield but also produced very little precipitate (less than 5 mg/l). When a base was not added to the solution, yield was much lower.

To determine if micronutrient concentrations in the recommended "CS" medium are near optimum, the trace element mixture was doubled and halved in an experiment summarized in Table 27. There was no significant difference between the treatments, although observations during the culture period indicated a slightly lower growth rate for the highest micronutrient concentrations.

Physical environmental factors

Factors other than nutrient media composition must be considered when specifying optimum conditions for the culture of an organism, for example, light intensity temperature, and availability of CO_2. Results from experiments to determine the sensitivity of _N. palea_ to changes in these factors are summarized in Table 28.

TABLE 25. RECOMMENDED CULTURE SOLUTION FOR Nitzschia palea. ("CS" MEDIUM)

Silicic acid solution[*]
  0.1325 g/l silicic acid (88.8% SiO_2)
  15 drops/l bromthymol blue
  5 ml/l 2N KOH or NaOH[†]
  Approx. 5 ml/l 2N HCl

Major nutrients

Salt	Stock solution, g/l	Final solution, ml stock/l	Concentration in final solution, ppm
$Ca(NO_3)_2 \cdot 4H_2O$[§]	69.16	10.0	N = 82
KH_2PO_4	3.08	10.0	Ca = 117
$MgSO_4 \cdot 7H_2O$	7.50	10.0	P = 7
Na_2CO_3	2.00	10.0	K = 9[#]
Fe Citrate[ξ]	0.60	10.0	Mg = 7.5
Citric acid[ϕ]	0.60	10.0	S = 9.7
EDTA 2Na	0.10	10.0	Fe = 1.12

Micronutrients[ϕ]

Salt	Stock solution, g/l	Final solution, ml stock/l	Concentration in final solution, ppm
KCl	3728	0.2	Cl = 0.35
H_2BO_3	1546	0.2	B = 0.054
$MnSO_4 \cdot H_2O$	845	0.2	Mn = 0.054
$ZnSO_4 \cdot 7H_2O$	575	0.2	Zn = 0.026
$CuSO_4 \cdot 5H_2O$	125	0.2	Cu = 0.006
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	18.4	0.2	Mo = 0.002
B_6 and C_{13} solutions		1.0 each	

[*] This solution is prepared in large quantities with a low silicon concentration and used in place of distilled water during media preparations. Solution containing silicic acid, indicator, and base is heated to 80°C, or allowed to stand for 1-2 days to dissolve the silicic acid. pH adjusted to 7.2 with the aid of bromthymol blue by addition of HCl.

[†] NaOH gives slightly higher N. palea yields. Experience proved this ratio of silicic acid to base works the best, although proportionally higher quantities of silicic acid can be dissolved in higher concentrations of base. The diatoms were able to tolerate up to 10 ml of 2N KOH, higher concentrations were not investigated.

[§] If a completely precipitate-free medium is desired, this stock solution should be autoclaved separately and added aseptically.

TABLE 25 continued

# Does not include KOH in silicic acid solution.

ξ Prepared as one stock solution and heated to 80°C to hasten solution.

ϕ Modified from Johnson, et al (1957).

Of the two temperatures investigated (24 and 19C), *N. palea* produced slightly more growth at 24C. This experiment was conducted in two controlled environment cabinets adjusted to provide a light intensity between 390 and 420 ft-c.

Nitzschia palea yields were not influenced by differences in the light intensities used in nutrition experiments (250-550 ft-c). However, higher intensities (650-700 ft-c) were slightly inhibitory.

Non-aerated cultures depend mainly on diffusion from the surrounding air for a supply of CO_2. The first aeration experiment showed that diffusion was not rapid enough to supply CO_2 for *N. palea* cultures. Yields from flasks with cotton plugs were significantly less than yields from flasks with aerators, even with a medium low in silicon. Enriching the air supply with CO_2 in the second experiment further increased yield.

Critical concentrations

The results from experiments to establish the critical concentrations for N, K, P, and Ca are summarized in Table 29.

In all experiments, the effects of a deficiency of an element were readily observable before harvest. This was especially true for the phosphorus experiment in which the diatoms not only showed an obvious

TABLE 26. COMPARISON OF SILICON SOURCES FOR Nitzschia palea.

Si source*	Precipitate, mg/l	Diatom yield,[†] mg/l	
		Replicates	Mean[§]
Sodium silicate	89	348,377,365,380	373 a
Silicic acid	< 5	221,224,229,227	225 b
Silicic acid dissolved in KOH[#]	< 5	414,394,450,398	416 c

[*] 25 ppm Si.

[†] Weight of precipitate subtracted from total dry weight values.

[§] Means with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

[#] 737 ppm K in final medium; pH adjusted with HCl.

difference in biomass but also in color, which ranged from yellow in deficient cultures to brown in cultures with adequate phosphorous. Because there has not been time for confirmatory experiments, approximate critical concentrations rather than specific values are presented.

DISCUSSION AND CONCLUSIONS

Because diatoms require relatively large amounts of silicon, establishing the critical concentrations for the essential nutrients in Nitzschia palea presented problems. High concentrations of $Na_2SiO_3 \cdot 9H_2O$ produced precipitates in culture media containing calcium and phosphorous salts.

TABLE 27. RESPONSE OF Nitzschia palea TO VARIOUS LEVELS OF THE ESSENTIAL TRACE ELEMENTS.

Basal medium	Trace element level	Mean yield,* mg/l
CS (Table 25)	0.5 x	676
	1.0 x[†]	670
	2.0 x	677

* Mean of four replicates; means with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

[†] Concentration in modified Johnson's micronutrients.

This caused errors in dry weight determinations and precluded the synthesis of a defined medium. The high silicon requirement also meant that N. palea might exhaust the supply of that element before the nutrient under investigation became limiting. The problem was compounded when $Ca(NO_3)_2$ produced higher yields than $NaNO_3$ or KNO_3.

These problems were solved in the development of the recommended medium. The primary difference between this medium and others reported in the literature for the culture of diatoms is that silicon is added as a solution of silicic acid instead of the conventional $Na_2SiO_3 \cdot 9H_2O$.

A vitamin requirement could not be demonstrated for N. palea, at least in the bacterized state. This is not surprising in view of the fact that species of N. palea have been isolated and cultivated since the late 1800's in purely inorganic media.

Nitzschia palea showed no obvious toxicity to the high concentrations of

TABLE 28. RESPONSE OF Nitzschia palea TO CHANGES IN VARIOUS PHYSICAL ENVIRONMENTAL FACTORS.

Physical environmental factor	Medium	No. of replicates	Treatment	Mean yield,* mg/l
Temperature	Low Si	6	19C	67 a
			24C	75 b
Light intensity	Low Si	6	250-300 ft-c	84 a
			500-550 ft-c	88 a
			650-700 ft-c	71 b
Aeration Experiment I	Low Si	6	Aeration	98 a
			No aeration	81 b
Aeration Experiment II	25 ppm Si, precipitate dissolved by HCl, and pH adjusted by NaOH	4	- CO_2	242 a
			+ CO_2	302 b

* Means with a common letter are not significantly different at the 5% level using Duncan's multiple range test.

sodium, potassium, or chlorine in the silicon solution. This conflicts somewhat with early work which showed a preference by diatoms for a monovalent/divalent ratio below 1.5 (Provasoli, 1958).

Because substitution of $Ca(NO_3)_2$ for $NaNO_3$ stimulated growth, the calcium salt is recommended as a N source even though the exact nature of the effect has not been determined. Early workers (Chu, 1942; Vollenweider 1950; and Provasoli, et al, 1954) reported a preference for a high calcium concentration in media for some freshwater diatoms and gave evidence for interaction between Ca, K, and Mg ions. In the present study, the stimulatory effect of $Ca(NO_3)_2$ on N. palea was not due to a high calcium requirement,

TABLE 29. CRITICAL CONCENTRATIONS AND RANGE OF CONCENTRATIONS OF FOUR ESSENTIAL NUTRIENT ELEMENTS IN *Nitzschia palea*.

Element	Critical concentration	Range in concentration
N	2.3 - 2.5%	1.49 - 4.56%
P	0.20 - 0.25%	0.07 - 0.61%
K	0.30 - 0.45%	0.28 - 0.89%
Ca	≤0.4%	-

nor was it the result of a toxicity due to high concentrations of sodium or potassium.

Caution must be exercised in comparing the critical concentrations presented in Table 29 to critical concentrations reported for other organisms. Because of a "dilution factor" caused by differences in the relative amounts of structural material in various organisms, comparisons of critical concentrations between species sometimes do not accurately reflect variations in protoplasmic requirements for an element. This would especially seem a problem with diatoms, whose cell walls not only have a unique structure and composition, but also exhibit large intraspecific differences in thickness. However, if the amount of cell wall material in the taxon remains relatively constant, then critical concentrations can provide information on nutritional requirements that are of ecological interest.

Of some concern is the observation by Lund (1950) and Lewin (1957) that cell wall thickness in diatoms varies with the division rate. Because of these reports, the ash content of samples of *N. palea* from various experiments was determined and was found to be a consistent 31%. This reflects a fairly constant SiO_2 percentage.

In comparison with values reported for other freshwater algae and aquatic angiosperms (Gerloff, 1975; and Gerloff and Fitzgerald, 1976), *N. palea* has an unusually high phosphorous critical concentration (0,20-0.25%). This range is typical of critical concentrations in index segments on terrestrial plants. An obvious question is the extent of the high phosphorous requirement among other diatoms. Although there have been many reports on the phosphorous concentration in diatoms which limited growth (Ketchum, 1939; Goldberg, et al, 1951; Mackereth, 1953; Quenzler and Ketchum, 1962; and Carpenter, 1970), these values were all expressed on a per cell basis. Values generally were between 5×10^{-14} and 2×10^{-15} grams P/cell. On the basis of data obtained in the present study, the limiting phosphorous concentration for *N. palea* was estimated to lie between 9.6 and 12.0×10^{-14} grams P/cell. Because *N. palea* cells are much smaller than most diatoms, the actual phosphorous requirement may be greater than this estimate. It is difficult to draw conclusions from comparisons on a per cell basis unless reliable information on cell size is available.

Although the critical concentration range for nitrogen in *N. palea* is higher than most values reported by Gerloff (1975) for freshwater algae and aquatic angiosperms, it is greatly exceeded by the critical concentration reported for *Microcystis aeruginosa* (4,0%). The critical concentration range for potassium in *N. palea* is slightly lower than the values given for most other aquatic organisms.

It would be interesting to determine if the high requirement for nitrogen and phosphorous shown by *Nitzschia palea* in these experiments, along with the rapid growth rate observed in the cultures, hinder the organism's ability to compete for these nutrients at the low concentrations present in the natural environment. It has long been observed that diatom blooms tend to form at the time of spring and fall turnover in lakes, when the level of nutrients is high, and then to fall off sharply after lake stratification.

SECTION 10

TESTS ON THE PRODUCTION BY ALGAE OF *Cladophora glomerata* GROWTH INHIBITORS

The production of inhibitors either toxic to the producing algae or to other algae has been demonstrated to be a rather common phenomenon under laboratory conditions (Harris, 1971; Fogg, 1975). It is, however, difficult to establish that these inhibitors are present in high enough concentrations to influence the presence and growth of algae under field conditions. Because a very potent inhibitor could be more critical than nutrient supply in controlling *Cladophora glomerata* occurrence, it seemed worthwhile to assay for the production of *C. glomerata* inhibitors by several algae species widely distributed in midwestern lakes.

EXPERIMENTAL PROCEDURES

Two procedures were used in the inhibitor tests. In one, two species were inoculated into a culture flask simultaneously and after a specific growth period the yield of each organism was determined. An inhibitory effect of one organism on another was indicated by comparisons with yields in cultures containing the individual species. Success in this approach required a nutrient medium in which both organisms would grow satisfactorily. It also was essential that one organism did not grow so much more rapidly than the other that reduced growth of the latter was due to restricted light supply or nutrients rather than inhibitor production. To avoid this possibility, C. glomerata, which grows rather slowly, was inoculated into cultures 5 to 7 days before the inhibitor organism.

The second procedure involved testing for the release into the culture medium during the growth of a specific alga of a substance inhibitory to C. glomerata. The organism tested was cultured in 200 ml of medium in 500 ml flasks using standard procedures. When heavy growth was observed, the cultures were filtered through an 8 μ millipore filter. Efforts then were made to grow C. glomerata in 40 ml volumes of the filtrate, with and without nutrient enrichment and autoclaving, in 21 x 150 mm test tubes. The solutions were vigorously aerated.

RESULTS

The data in Tables 30 through 32 show the capacity of Cladophora glomerata to grow in the presence of two common unicellular green algae, Scenedesmus quadricauda and a species of Chlorella. The first two treatments show yields of a pair of organisms when grown separately; the third indicates the yield of each organism after the two were inoculated into a culture at the same time and grew together.

A decreased C. glomerata yield could result from extremely rapid growth of a competing organism which overwhelmed C. glomerata in competition for light and nutrients. To recognize this possibility, in the fourth treatment the competing organism was introduced into the culture 7 days after the C. glomerata.

As shown in Tables 30 and 31, the Chlorella species and Scenedesmus quadricauda from Lake Michigan were without major effect on Cladophora glomerata growth. There was a marked reduction in yield of C. glomerata when both organisms were inoculated together. However, when C. glomerata

TABLE 30. EFFECT OF LAKE MICHIGAN Scenedesmus quadricauda ON GROWTH OF Cladophora glomerata IN A COMBINED CULTURE

Treatment*	Ave. dry-wt. yield, mg/l	
	Scenedesmus	Cladophora
Cladophora only	---	295
Scenedesmus only	462	---
Clad. & Scen. both inoc. at start	469	44
Scenedesmus inoc. 7 days after Clad.	122	223
Clad. after 7 day culture period	---	42

* The culture solution was the Cladophora medium with the N increased to 32.4 ppm and P to 7 ppm; 14 day culture period; 450-500 ft-c.

was inoculated 7 days prior to the green algae, the C. glomerata continued to grow throughout the culture period.

The results with a species of Scenedesmus quadricauda isolated from Lake Mendota were somewhat different (Table 32), Cladophora glomerata growth was completely inhibited when the two organisms were inoculated at the same time; growth also was severely inhibited when the S. quadricauda was inoculated a week after the C. glomerata. In fact, there was almost no growth following the introduction of the S. quadricauda. The production of a C. glomerata inhibitor is strongly suggested.

The data in Table 33 are from experiments in which efforts were made to grow C. glomerata in the filtrate from cultures of several common Great

TABLE 31. EFFECT OF LAKE MICHIGAN Chlorella sp. ON GROWTH OF Cladophora glomerata IN A COMBINED CULTURE

Treatment*	Ave. dry-wt. yield, mg/l	
	Chlorella	Cladophora
Cladophora only	---	421
Chlorella only	592	---
Clad. & Chlorella both inoc. at start	623	165
Chlorella inoc. 7 days after Clad.	133	383
Clad. after 7 day culture period	---	67

* The culture solution was the Cladophora medium with the N increased to 32.4 ppm and P to 7 ppm; 14 day culture period; 450-500 ft-c.

Lakes algae. The critical data are in the columns indicating growth in the inhibitor organism culture filtrate after various treatments. For comparison purposes, growth in the standard Cladophora medium also is indicated. Failure of the C. glomerata to grow in the filtrate after passage through an 8 millipore filter is of little significance, because the nutrients in the medium could have been depleted by the inhibitor organism. Growth in filtrate on nutrient-enriched filtrate approximating that in autoclaved Cladophora medium indicates an inhibitor was not produced. This was true for Scenedesmus dimorphus, in two tests with Selenastrum gracile, with the Chlamydomonas sp., and with the diatom Nitzschia palea.

Initially the restoration of the potential for C. glomerata growth when the nutrient-enriched medium was autoclaved was interpreted as due

TABLE 32. EFFECT OF LAKE MENDOTA *Scenedesmus quadricauda* ON GROWTH OF *Cladophora glomerata* IN A COMBINED CULTURE

Treatment*	Ave. dry-wt. yield, mg/l	
	Scenedesmus	*Cladophora*
Scenedesmus only	620	---
Cladophora only	---	435
Clad. & *Scen.*, both inoc. at start	717	6
Scenedesmus inoc. 7 days after *Clad.*	527	69

* The culture solution was the *Cladophora* medium with the N increased to 32.4 ppm and P to 7 ppm; 21 day culture period; 500-600 ft-c.

to heat destruction of an inhibitor produced by an organism from the left column. This would be consistent with the results obtained in other investigations. However, another explanation is possible and cannot be eliminated. The autoclaving could inactivate some of the nutrients which reached toxic levels as a result of enrichment of the filtrates. The results suggest production of a heat-labile inhibitor by *Scenedesmus quadricauda* but until further experiments are carried out the alternative explanation is a possibility. The differences in the results in the two experiments with the same *Scenedesmus* species perhaps was associated with the use of different culture media.

DISCUSSION

The preliminary results on algae production of compounds inhibitory to *Cladophora glomerata* were comparable to results from studies with other

TABLE 33. TESTS ON THE PRODUCTION BY VARIOUS ALGAE ON *Cladophora glomerata* GROWTH INHIBITOR

Algae	Yield of inhibitor inoculum culture, mg/1	Ave. dry wt. *Cladophora* inoculum, mg	Ave. yield of *Cladophora*, mg/1			
				Inhibitor organism culture filtrate		
			Autoclaved *Cladophora* medium*	8µ MPF	8µ MPF + nutrients	8µ MPF +nutrients +autoclave
Scenedesmus quadricauda†	-	-	368	85	0	376
Scenedesmus quadricauda†	240	1.3	398	148	189	362
Scenedesmus bijuga	443	2.2	178	89	191	192
Scenedesmus dimorphus	300	2.5	269	249	0	232
Selenastrum gracile	-	2.6	214	215	240	205
Selenastrum gracile	200	2.6	184	168	162	238
Chlamydomonas sp.	250	2.3	209	177	126	131
Chlorella sp.	282	1.7	233	124	60	198
Chlorella sp.†	735	1.3	270	159	101	208
Nitzschia palea†	443	2.0	285	317	97	383

TABLE 33. (continued)

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* Culture period varied from 7-14 days.

† The culture solution for both *Cladophora* and the inhibitor organism was *Cladophora* medium except in the second *S. quadricauda* and *Chlorella* sp. experiments in which Gorham's Medium was used, and in *Nitzschia palea* in which Gorham's Medium with increased silicon was used.

algae. It has been relatively simple to obtain laboratory data which suggest inhibitor production occurs. However, laboratory studies for the most part have involved inhibitor assays in small-volume cultures in which organisms were present in far greater numbers than even in major blooms. The possibilities for dilution of any natural inhibitor under lake conditions make it doubtful that inhibitors are a significant ecological factor.

From the present study, the common unicellar green alga Scenedesmus quadricauda seems the most promising organism for any further inhibitor investigations. However, the evidence forproduction of a highly toxic inhibitor was limited and, as with other algae, the possibility it is important under field conditions seems slight.

SECTION 11

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SECTION 12

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APPENDIX A

STATISTICAL ANALYSES

TABLE A-1. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT GREEN BAY CAMPUS JULY 26, 1976

Homoscedastic group	Treatment within homoscedastic group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A Cladophora medium	562	c
	B Clad. medium in l.w.	793	d
	D As B, - vit. B_{12}	435	bc
	G As B, - Fe	140	a
	J As B, - B, low B glass	396	bc
	K As B, - K	319	b
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	749	d
II	C As B, - vit. B_1	82	a
	E As B, - N	87	a
	F As B, - P	140	b
	H As B, - S	704	b
	I As B, - B, pyrex	818	b

(continued)

TABLE A-1. (continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/l	EMHV†
I	B *Clad*. med. in l.w./C As B, - vit. B_1	793/82	SD
II	B *Clad*. med. in l.w./E As B, - N	793/87	SD
III	B *Clad*. med. in l.w./F As B, - P	793/140	SD
IV	B *Clad*. med. in l.w./H As B, - S	793/704	SD
V	B *Clad*. med. in l.w./I As B. - B, pyrex	793/818	NS
VI	G As B, - Fe/C As B, - vit. B_1	140/82	NS
VII	G As B, - Fe/E As B, - N	140/87	NS
VIII	G As B, - Fe/F As B, - P	140/140	NS
IX	J As B, + B, low B glass/I As B, - B, pyrex	396/818	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-2. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT GREEN BAY CAMPUS SEPTEMBER 12, 1976

Homoscedastic group	Treatment within homoscedastic group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	584	a
	B *Clad*. medium in l.w.	620	a
	D As B, - vit. B_{12}	556	a
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	538	a
	O As M, - vit. B_{12}	638	a
II	C As B, - vit. B_1	132	b
	E As B, - N	141	b
	K As B, - K	271	c
	N As M, - vit. B_1	82	a
III	F As B, - P	287	a
	G As B, - Fe	249	a
	H As B, - S	686	c
	J As B, low B glass	356	a
	M As B, filtered l.w. + vit.	773	c
	P As M, - Fe	461	b

(continued)

TABLE A-2 (continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/l	EMHV[†]
I	B Clad. med. in l.w./C As B, - vit. B_1	620/132	SD
II	B Clad. med. in l.w./E As B, - N	620/141	SD
III	B Clad. med. in l.w./F As B, - P	620/287	SD
IV	B Clad. med. in l.w./G As B, - Fe	620/249	SD
V	B Clad. med. in l.w./H As B, - S	620/686	NS
VI	B Clad. med. in l.w./J As B, low B glass	620/356	SD
VII	B Clad. med. in l.w./K As B, - K	620/271	SD
VIII	B Clad. med. in l.w./M As B, filt. l.w. + vit.	620/773	NS
IX	B Clad. med. in l.w./N As M, - vit B_1	620/82	SD
X	B Clad. med. in l.w./P As M, - Fe	620/461	NS
XI	E As B, - N/ F As B, - P	141/287	SD
XII	E As B, - N/G As B, - Fe	141/249	SD
XIII	C As B, - vit. B_1/F As B, - P	132/287	SD

(continued)

TABLE A-2. (continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/1	EMHV[†]
XIV	C As B, - vit. B_1/G As B, - Fe	132/249	SD

[*] Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

[†] NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-3. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT BAYSHORE PARK JULY 26, 1976

Homoscedastic group	Treatment within homoscedastic group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	649	bc
	B *Clad*. medium in l.w.	778	c
	D As B, - vit. B_{12}	324	a
	F As B, - P	164	a
	I As B, - B, pyrex	869	c
	J As B, - B, low B glass	420	ab
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	709	c
II	C As B, - vit. B_1	71	a
	E As B, - N	52	a
	G As B, - Fe	155	b
	H As B, - S	501	b
	K As B, - K	280	b

(continued)

TABLE A-3. (continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/1	EMHV†
I	B *Clad.* med. in 1.w./C As B, - vit. B_1	778/71	SD
II	B *Clad.* med. in 1.w./E As B, - N	778/52	SD
III	B *Clad.* med. in 1.w./G As B, - Fe	778/155	SD
IV	B *Clad.* med. in 1.w./H As B, - S	778/501	SD
V	B *Clad.* med. in 1.w./K As B, - K	778/280	SD
VI	F As B, - P/C As B, - vit. B_1	164/71	SD
VII	F As B, - P/E As B, - N	164/52	SD
VIII	F As B, - P/ G As B, - Fe	164/155	NS

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-4. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT BAYSHORE PARK AUGUST 15, 1976

Homogeneous group	Treatment within homogeneous group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	494	a
	B *Clad*. medium in l.w.	586	ab
	D As B, - vit. B_{12}	706	b
	J As B, - B, low B glass	338	c
	M As B, filtered l.w. + vit.	718	b
II	C As B, - vit. B_1	134	a
	E As B, - N	73	b
	F As B, - P	177	c
	G As B, - Fe	124	a
	N As M, - vit. B_1	169	c

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

TABLE A-5. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT BAYSHORE PARK SEPTEMBER 12, 1976

Homoscedastic group	Treatment within homoscedastic group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	564	b
	B *Clad*. medium in l.w.	761	c
	D As B, - vit. B_{12}	525	b
	F As B, - P	190	a
	H As B, - S	476	b
	J As B, - B, low B glass	430	b
	M As B, filtered l.w. + vit.	543	b
	O As M, - vit. B_{12}	458	b
II	C As B, - vit. B_1	80	a
	E As B, - N	94	a
	G As B, - Fe	102	a
	K As B, - K	240	b
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	254	b
	N As M, - vit. B_1	75	a
	P As M, - Fe	70	a

(continued)

TABLE A-5. (continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/1	EMHV†
I	B *Clad*. med. in 1.w./C As B, - vit. B_1	761/80	SD
II	B *Clad*. med. in 1.w./E As B, - N	761/94	SD
III	B *Clad*. med. in 1.w./G As B, - Fe	761/102	SD
IV	B *Clad*. med. in 1.w./K As B, - K	761/240	SD
V	B *Clad*. med. in 1.w./L As B, - Ca, Mg, Mn, Mo, Zn, Cu	761/254	SD
VI	B *Clad*. med. in 1.w./N As M, - vit. B_1	761/75	SD
VII	B *Clad*. med. in 1.w./P As M, - Fe	761/70	SD
VIII	F As B, - P/C As B, - vit. B_1	190/80	SD
IX	F As B, - P/E As B, - N	190/94	SD
X	F As B, - P/G As B, - Fe	190/102	NS

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-6. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT EGG HARBOR JULY 25, 1976

Homoscedastic group	Treatment within homoscedastic group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	597	b
	B *Clad*. medium in l.w.	660	b
	C As B, - vit. B_1	107	a
	H As B, - S	543	b
	I As B, - B, pyrex	713	b
	J As B, - B, low B glass	545	b
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	580	b
II	E As B, - N	21	a
	F As B, - P	64	b
	G As B, - Fe	72	b
	K As B, - K	200	c

(continued)

TABLE A-6. (continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/l	EMHV†
I	B *Clad*. med. in l.w./E As B, - N	660/21	SD
II	B *Clad*. med. in l.w./F As B, - P	660/64	SD
III	B *Clad*. med. in l.w./G As B, - Fe	660/72	SD
IV	B *Clad*. med. in l.w./K As B, - K	660/200	SD
V	C As B, - vit. B_1/E As B, - N	107/21	SD
VI	C As B, - vit. B_1/F As B, - P	107/64	NS
VII	C As B, - vit. B_1/G As B, - Fe	107/72	NS

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-7. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT EGG HARBOR SEPTEMBER 12, 1976

Homoscedastic group	Treatment within homoscedastic group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	690	ef
	B *Clad*. medium in l.w.	764	f
	D As B, - vit. B_{12}	606	de
	G As B, - Fe	288	b
	H As B, - S	493	cd
	J As B, - B, low B glass	342	b
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	565	de
	M As B, filtered l.w. + vit.	539	de
	O As M, - vit. B_{12}	389	bc
	P As M, - Fe	142	a
II	C As B, - vit. B_1	76	a
	E As B, - N	86	a
	F As B, - P	149	b
	K As B, - K	220	b
	N As M, - vit B_1	44	c

(continued)

TABLE A-7.(continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/1	EMHV†
I	B *Clad*. med. in 1.w./C As B, - vit. B_1	764/76	SD
II	B *Clad*. med. in 1.w./E As B, - N	764/86	SD
III	B *Clad*. med. in 1.w./F As B, - P	764/149	SD
IV	B *Clad*. med. in 1.w./K As B, - K	764/220	SD
V	B *Clad*. med. in 1.w./N As M, - vit. B_1	764/44	SD
VI	G As B, - Fe/C As B, - vit. B_1	288/76	SD
VII	G As B, - Fe/E As B, - N	288/86	SD
VIII	G As B, - Fe/F As B, - P	288/149	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-8. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT GILL'S ROCK JULY 25, 1976

Homoscedastic group	Treatment within homoscedastic group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	840	ab
	D As B, - vit. B_{12}	566	c
	F As B, - P	181	d
	I As B, - B, pyrex	920	a
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	712	bc
II	B *Clad.* medium in l.w.	1038	a
	C As B, - vit. B_1	239	b
	E As B, - N	43	c
	G As B, - Fe	152	d
	H As B, - S	458	e
	J As B, - B, low B glass	578	f
	K As B, - K	350	g

(continued)

TABLE A-8. (continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/l	EMHV†
I	B *Clad*. med. in l.w./A *Clad*. medium	1038/840	SD
II	B *Clad*. med. in l.w./D As B, - vit. B_{12}	1038/566	SD
III	B *Clad*. med. in l.w./F As B, - P	1038/181	SD
IV	B *Clad*. med. in l.w./I As B, - B, pyrex	1038/920	NS
V	B *Clad*. med. in l.w./L As B, - Ca, Mg, Mn, Mo, Zn, Cu	1038/712	SD
VI	F As B, - P/C As B, - vit. B_1	181/239	NS
VII	F As B, - P/E As B, - N	181/43	SD
VIII	F As B, - P/G As B, - Fe	181/152	NS
IX	I As B, - B, pyrex/J As B, - B, low B glass	920/578	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-9. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT GILL'S ROCK AUGUST 15, 1976

Homoscedastic group	Treatment within homoscedastic group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	635	c
	C As B, - vit. B_1	81	ab
	E As B, - N	130	b
	F As B, - P	43	a
	G As B, - Fe	85	ab
	H As B, - S	473	c
	K As B, - K	228	c
	O As N, - vit. B_1	57	a
II	B *Clad* medium in l.w.	829	b
	D As B, - vit. B_{12}	381	a
	M As B, - Mn	784	b
	J As B, - B, low B glass	415	a
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	866	b
	N As B, filtered l.w. + vit.	680	b

(continued)

TABLE A-9. (continued)

Heteroscedastic group	Treatment within heteroscedastic group	Ave. algae dry-wt. yield, mg/l	EMHV†
I	B Clad. med. in l.w./A Clad. medium	829/635	SD
II	B Clad. med. in l.w./C As B, - vit. B_1	829/81	SD
III	B Clad. med. in l.w./E As B, - N	829/130	SD
IV	B Clad. med. in l.w./F As B, - P	829/43	SD
V	B Clad. med. in l.w./G As B, - Fe	829/85	SD
VI	B Clad. med. in l.w./H As B, - S	829/473	SD
VII	B Clad. med. in l.w./K As B, - K	829/228	SD
VIII	B Clad. med. in l.w./O As N, - vit. B_1	829/57	SD

* Yield with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-10. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT GILL'S ROCK SEPTEMBER 12, 1976

Homogeneous group	Treatment within homogeneous group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	742	c
	B *Clad*. medium in l.w.	741	c
	C As B, - vit. B_1	103	a
	D As B, - vit. B_{12}	665	c
	F As B, - P	185	ab
	H As B, S	665	c
	J As B, - B, low B glass	742	c
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	582	c
	N As B, filtered l.w. + vit.	806	c
	O As N, - vit. B_1	329	b
	P As N, - vit. B_{12}	631	c
II	E As B, - N	66	a
	G As B, - Fe	81	a
	K As B, - K	231	b
	Q As N, - Fe	59	a

(continued)

TABLE A-10. (continued)

Heterogeneous group	Treatment within heterogeneous group	Ave. algae dry-wt. yield, mg/l	EMHV[†]
I	B *Clad*. med. in l.w./E As B, - N	741/66	SD
II	B *Clad*. med. in l.w./G As B, - Fe	741/81	SD
III	B *Clad*. med. in l.w./K As B, - K	741/231	SD
IV	B *Clad*. med. in l.w./Q As N, - Fe	741/59	SD
V	C As B, - vit. B_1/E As B, - N	103/66	NS
VI	C As B, - vit. B_1/G As B, - Fe	103/81	NS
VII	F As B, - P/E As B, - N	185/66	NS
VIII	F As B, - P/G As B, - Fe	185/81	NS

[*] Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

[†] NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-11. STATISTICAL ANALYSES OF YIELD OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT BAILEY'S HARBOR JULY 25, 1976

Homogeneous group	Treatment within homogeneous group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	821	cd
	B *Clad.* medium in l.w.	912	d
	D As B, - vit. B_{12}	720	bcd
	G As B, - Fe	146	a
	I As B, - B, pyrex	907	d
	J As B, - B, low B glass	584	bc
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	543	b
II	C As B, - vit. B_1	58	a
	E As B, - N	42	a
	F As B, - P	84	a
	H As B, - S	593	b
	K As B, - K	213	b

(continued)

TABLE A-11. (continued)

Heterogeneous group	Treatment within heterogeneous group	Ave algae dry-wt. yield, mg/l	EMHV†
I	B *Clad*. med. in l.w./C As B, - vit. B_1	912/58	SD
II	B *Clad*. med. in l.w./E As B, - N	912/42	SD
III	B *Clad*. med. in l.w./F As B, - P	912/84	SD
IV	B *Clad*. med. in l.w./H As B, - S	912/593	SD
V	B *Clad*. med. in l.w./K As B, - K	912/213	SD
VI	G As B, - Fe/C As B, - vit. B_1	146/58	NS
VII	G As B, - Fe/E As B, - N	146/42	SD
VIII	G As B, - Fe/F As B, - P	146/84	NS

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-12. STATISTICAL ANALYSES OF YIELDS OF *Cladophora glomerata* RESPONSE TO DIFFERENTIAL NUTRIENT ENRICHMENT OF LAKE MICHIGAN WATER COLLECTED AT BAILEY'S HARBOR SEPTEMBER 12, 1976

Homogeneous group	Treatment within homogeneous group	Ave. algae dry-wt. yield, mg/l	SNK*
I	A *Cladophora* medium	653	c
	B *Clad*. medium in l.w.	763	c
	D As B, - vit. B_{12}	433	b
	J As B, - B, low B glass	346	b
	L As B, - Ca, Mg, Mn, Mo, Zn, Cu	465	b
	M As B, filtered l.w. + vit.	388	b
	O As M, - vit. B_{12}	123	a
II	C As B, - vit. B_1	58	ab
	E As B, - N	58	ab
	F As B, - P	156	c
	G As B, - Fe	84	b
	H As B, - S	505	c
	K As B, - K	201	c
	N As M, - vit. B_1	41	ab
	P As M, - Fe	8	a

(continued)

TABLE A-12. (continued)

Heterogeneous group	Treatment within heterogeneous group	Ave. algae dry-wt. yield, mg/1	EMHV†
I	B *Clad*. med. in 1.w./C As B, - vit. B_1	763/58	sd
II	B *Clad*. med. in 1.w./E As B, - N	763/58	SD
III	B *Clad*. med. in 1.w./F As B, - P	763/156	SD
IV	B *Clad*. med. in 1.w./GAs B, - Fe	763/84	SD
V	B *Clad*. med. in 1.w./HAs B, - S	763/505	SD
VI	B *Clad*. med. in 1.w./KAs B, - K	763/201	SD
VII	B *Clad*. med. in 1.w./NAs M, - vit. B_1	763/41	SD
VIII	B *Clad*. med. in 1.w./PAs M, - Fe	763/8	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-13. STATISTICAL ANALYSES ON THE YIELD IN THE FITZGERALD TEST FOR HOT WATER EXTRACTABLE P IN *Cladophora glomerata*

Homoscedastic group	Treatment in group, P in culture soln., ppm	Ave. algae dry-wt. yield, mg/l	SNK*
I	0.07	221	a
	0.14	342	b
	0.28	414	c
II	0.56	546	a
	1.12	625	a
	2.80	589	a

Heteroscedastic group	Treatment in group, P in culture soln., ppm	Ave. algae dry-wt. yield, mg/l	EMHV†
I	0.28/0.56	414/546	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-14. STATISTICAL ANALYSES ON THE YIELD OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN N CONCENTRATION AND CULTURE PERIOD

Homoscedastic group	Treatment in group, N added to culture soln., ppm	Ave. algae dry-wt. yield, mg/l	SNK*
I 14 day culture period	1.0	96	a
	2.0	209	b
II 14 day culture period	4.0	394	a
	6.0	423	a
III 14 day culture period	8.2	386	a
	16.4	344	a
I 21 day culture period	1.0	120	a
	2.0	264	b
	4.0	429	c
II 21 day culture period	6.0	565	a
	8.2	660	b
	16.4	708	b

(continued)

TABLE A-14(continued)

Heteroscedastic group	Treatment in group, N added to culture soln., ppm	Ave. algae dry-wt. yield, mg/l	EMHV†
14 day culture period			
I	2.0/4.0	209/394	SD
II	6.0/8.2	423/386	NS
21 day culture period			
I	4.0/6.0	429/565	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-15. STATISTICAL ANALYSES ON THE YIELD OF Cladophora glomerata GROWN IN CULTURE MEDIUM DIFFERING IN P CONCENTRATION AND CULTURE PERIOD

Homoscedastic group	Treatment in group, P added to culture soln., ppm	Ave. algae dry-wt. yield, mg/l	SNK*
I	.07	88	a
14 day	.14	230	b
culture	.28	266	b
period	.56	307	c
	1.12	353	c
	2.80	343	c
I			
21 day	.07	117	a
culture	.28	377	b
period	.56	435	b
	1.12	632	c
	2.80	509	b

Heteroscedastic group	Treatment in group, P added to culture soln., ppm	Ave. algae dry-wt. yield, mg/l	EMHV†
21 day culture period			
I	.07/.14	117/282	SD
II	.14/.28	282/377	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-16. STATISTICAL ANALYSES ON THE YIELD OF Cladophora glomerata AND THE EFFECT OF DIFFERENT PATTERNS OF ADDITION OF N

Homoscedastic group	Treatment in group	Ave. algae dry-wt. yield, mg/l	SNK*
I	16.4 ppm N, initially	311	a
	1.6 ppm N, initially	219	b
II	0.8 ppm N, initially; at 9 days, .16 ppm N each of 5 days	172	a
	10 additions of .16 ppm N during 14 day period	160	a

Heteroscedastic group	Treatment in group	Ave. algae dry-wt. yield, mg/l	EMHV†
I	16.4 ppm N, initially/0.8 ppm N, etc.	311/172	SD
II	16.4 ppm N, initially/10 additions, etc.	311/160	SD
III	1.6 ppm N, initially/0.8 ppm N, etc.	219/172	SD
IV	1.6 ppm N, initially/ 10 additions, etc.	219/160	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-17. STATISTICAL ANALYSES ON THE YIELD OF *Cladophora rivularis* ISOLATED FROM LAKE HURON GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

Homoscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	SNK*
I	1.21	149	a
	1.90	131	a
	2.90	169	a
	4.85	270	b
II	2.43	184	a
	9.7	554	b
	14.6	542	b
	19.4	494	b

Heteroscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	EMHV†
I	1.90/2.43	131/184	NS
II	2.43/2.90	184/169	NS
III	4.85/9.7	270/554	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-18. STATISTICAL ANALYSES ON THE YIELD OF *Cladophora glomerata* ISOLATED FROM LAKE ERIE GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

Homoscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	SNK*
I	1.21	96	a
	1.90	168	b
	2.43	212	c
	2.90	238	d
II	4.85	362	a
	9.7	468	a
	14.6	475	a
	19.4	445	a

Heteroscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	EMHV†
I	2.90/4.85	238/362	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-19. STATISTICAL ANALYSES ON THE YIELD OF *Cladophora glomerata* ISOLATED FROM LAKE ONTARIO GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

Homoscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	SNK*
I	1.21	183	a
	1.90	206	a
	2.43	196	a
	2.90	221	a
	4.85	431	b
	14.60	641	b
II	9.70	659	a
	19.4	653	a

Heteroscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	EMHV†
I	4.85/9.7	431/659	SD
II	9.7/14.6	659/641	NS
III	14.6/19.4	641/653	NS

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-20. STATISTICAL ANALYSES ON THE YIELD OF *Draparnaldia plumosa* GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

Homoscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	SNK*
I	0.12	173	c
	0.24	196	c
	0.49	306	c
	0.97	466	b
	1.94	702	a
	4.85	598	a
II	9.7	827	a
	19.4	778	a

Heteroscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	EMHV†
I	4.85/9.7	598/827	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means for heterogeneous variances test.

TABLE A-21. STATISTICAL ANALYSES ON THE YIELD OF *Nostoc muscorum* GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

Homoscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	SNK[*]
I	0.12	115	d
	0.24	192	c
	0.49	284	b
	4.85	258	a
II	0.97	249	a
	1.94	243	a
	9.70	245	a
	19.40	230	a

Heteroscedastic group	Treatment in group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	EMHV[†]
I	4.85/9.7	258/245	NS
II	1.94/4.85	243/258	NS
III	0.49/0.97	284/249	NS

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[*] Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

[†] NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-22. STATISTICAL ANALYSIS ON THE YIELD OF *Microcystis aeruginosa* GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

Homoscedastic group, S added to culture solution, ppm	Ave. algae dry-wt. yield, mg/l	SNK*
0.12	54	c
0.24	61	c
0.49	166	b
0.97	203	a
1.94	207	a
4.85	192	ab
9.70	207	a
19.40	177	ab

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

TABLE A-23. STATISTICAL ANALYSES ON THE YIELD OF *Cladophora glomerata* IN RESPONSE TO THE ADDITION OF C_{13} MICRONUTRIENTS USING ZOOSPORES AS INOCULUM

Homoscedastic group	Treatment in group	Ave. algae dry-wt. yield, mg/l	SNK*
I	*Clad*. medium, plus C_{13}	741	a
	Clad. medium, minus As	563	a
	Clad. medium, minus Cd	674	a
	Clad. medium, minus Hg	776	a
	Clad. medium, minus Pb	783	a
	Clad. medium, minus F	778	a
	Clad. medium, minus Se	626	a
	Clad. medium, minus Be	736	a
II	*Clad*. medium, minus C_{13}	12	a
	Clad. medium, minus C_{13} "7"	14	a

Heteroscedastic group	Treatment in group	Ave. algae dry-wt. yield, mg/l	EMHV†
I	Plus C_{13}/minus C_{13}	741/12	SD
II	Plus C_{13}/minus C_{13} "7"	741/14	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

TABLE A-24. STATISTICAL ANALYSES ON THE YIELD RESPONSE OF *Cladophora glomerata* TO VARIOUS CONCENTRATIONS OF MANGANESE

Homoscedastic group	Treatment in group, Mn added to culture soln., ppm	Ave. algae dry-wt. yield, mg/l	SNK*
I	0.00	356	a
	2.16	342	a
	2.43	296	a
	2.70	188	b
II	0.27	698	a
	1.35	606	a

Heteroscedastic group	Treatment in group, Mn added to culture soln., ppm	Ave. algae dry-wt. yield, mg/l	EMHV†
I	0.00/0.27	356/698	SD
II	1.35/2.16	606/342	SD

* Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

† NS = not significant at the 5% level; SD = significantly different at the 5% level using the equality of means with heterogeneous variances test.

APPENDIX B

ADDITIONAL EXPERIMENTAL DATA

TABLE B-1. STANDARDIZATION OF FITZGERALD TEST FOR HOT WATER EXTRACTABLE PHOSPHORUS IN *Cladophora glomerata*

P in culture soln.,* ppm	Ave. algae dry-wt yield,† mg/l	Extractable P, %
0.07	221	0.005
0.14	342	0.007
0.28	414	0.008
0.56	546	0.011
1.12	625	0.013
2.80	589	0.042

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 and C_{13} solutions, vitamins B_1 and B_{12} and Na_2CO_3 and Na_2SiO_3.

† Average of three replicates per treatment; 21-day culture period.

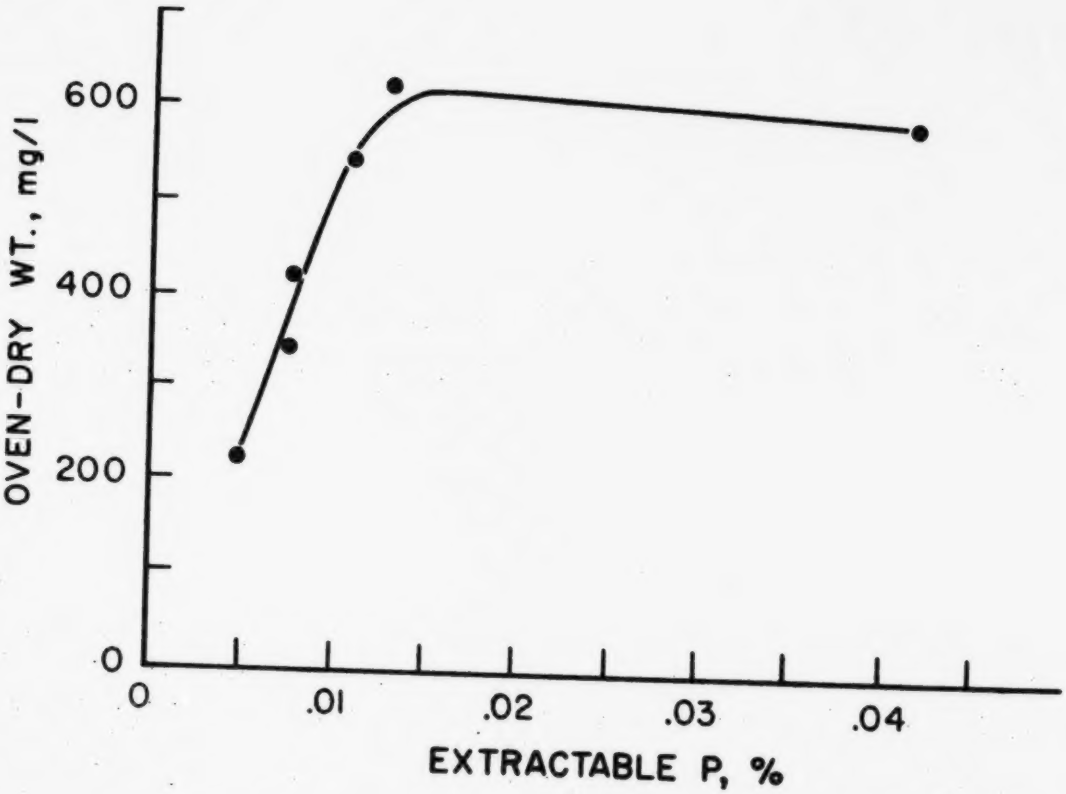

FIGURE B-1. Relationship between yield and hot-water extractable P in *Cladophora glomerata* cultured under various levels of P.

TABLE B-2. STANDARDIZATION OF ALKALINE PHOSPHATASE TEST FOR PHOSPHORUS AVAILABILITY TO *Cladophora glomerata*

P in culture soln.,* ppm	Ave. algae dry-wt,† mg/l	Phosphatase activity, §
.035	278	304
.07	238	309
.14	291	353
.28	433	246
.56	515	136
1.12	558	62
2.80	706	12

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 and C_{13} solutions, vitamins B_1 and B_{12}, and Na_2CO_3 and Na_2SiO_3.

† Average of three replicates per treatment.

§ One unit of alkaline phosphatase activity equals the amount of enzyme which liberates one millimicromole of nitrophenol/hr, under the prescribed conditions.

TABLE B-3. STANDARDIZATION OF NH_4-N UPTAKE TEST TO INDICATE NITROGEN NUTRITION OF *Cladophora glomerata*

N in culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	NH_4-N uptake, µg N/10 mg algae/hr	SNK§
1.5	313	22.5	a
2.5	366	32.1	ab
4.0	411	34.9	abc
6.0	472	31.7	abc
10.0	569	29.4	c
16.4	510	24.6	bc
32.4	491	15.0	abc

[*] The nutrient medium contained 2.8 ppm P, 23.4 ppm Ca, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 and C_{13} solutions, vitamins B_1 and B_{12}, and Na_2CO_3 and Na_2SiO_3.

[†] Average of two replicates per treatment; 22-day culture period.

[§] Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

TABLE B-4. STANDARDIZATION OF NITRATE ACCUMULATION TEST TO INDICATE NITROGEN NUTRITION OF *Cladophora glomerata*

N in culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	NO_3 in algae, ppm
1.5	280	14
2.5	387	85
4.0	455	140
8.0	700	96
16.4	747	97
24.6	742	126
32.8	606	692

* The nutrient medium contained 2.8 ppm P, 23.4 ppm Ca, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, B_7 and C_{13} solutions, vitamins B_1 and B_{12}, and Na_2CO_3 and Na_2SiO_3.

† Average of two replicates per treatment.

TABLE B-5. THE EFFECT OF CULTURE PERIOD LENGTH ON N CRITICAL CONCENTRATION IN *Cladophora glomerata*

	14-day culture period		21-day culture period	
N added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	Ave. P conc. in algae, %	Ave. algae dry-wt. yield,† mg/l	Ave. P conc. in algae, %
1.0	96	.89	120	.77
2.0	209	.79	264	.67
4.0	394	.80	429	.75
6.0	423	1.09	565	.64
8.2	386	1.27	660	.97
16.4	344	2.85	708	1.65

* The nutrient medium contained 23.4 ppm Ca, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, and Na_2CO_3 and Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

† Average of five replicates in each treatment.

TABLE B-6. THE EFFECT OF CULTURE PERIOD LENGTH ON P CRITICAL CONCENTRATION IN *Cladophora glomerata*

	14-day culture period		21-day culture period	
P added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	Ave. P conc. in algae, %	Ave. algae dry-wt. yield,† mg/l	Ave. P conc. in algae, %
0.07	88	.07	117	.05
0.14	230	.05	282	.04
0.28	266	.09	377	.05
0.56	307	.07	435	.18
1.12	353	.26	632	.14
2.80	343	.64	509	.43

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 18 ppm K, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace elements, Na_2CO_3 and Na_2SiO_3, B_7 and C_{13} solutions, and vitamin B_1 and B_{12}.

† Average of five replicates in each treatment.

TABLE B-7. THE EFFECT OF LOW K CONCENTRATION ON P CRITICAL CONCENTRATION IN *Cladophora glomerata*

P added to culture soln.,[*] ppm	Ave. algae dry wt. yield,[†] mg/l	Ave. P conc. in algae, %	SNK[§]
.028	102	.05	a
.042	121	.03	a
.07	122	---	a
.14	164	.06	b
.28	259	.07	c
.56	292	.13	c
1.12	295	.29	c
2.80	255	.39	c

[*] K level in all treatments was 1.8 ppm; the nutrient medium contained 16.4 ppm N, 23.4 ppm Ca, 7.5 ppm Mg, 9.7 ppm S, 1.12 ppm Fe, 1/5 Johnson's trace, Na_2CO_3 and Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

[†] Average of five replicates in each treatment; 17-day culture period.

[§] Yields with a common letter are not significantly different at the 5% level using Student-Newman-Keuls multiple range test.

TABLE B-8. YIELD AND S CONCENTRATION IN *Cladophora rivularis* ISOLATED FROM LAKE HURON GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

S added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	Ave. S conc. in algae, %
1.21	149	0.90
1.90	131	1.26
2.43	184	1.54
2.90	169	1.62
4.85	270	1.41
9.7	554	1.80
14.6	542	2.28
19.4	494	3.46

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 1.12 ppm Fe, 1/5 Johnson's trace elements, Na_2CO_3, Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

† Average of five replicates in each treatment; 22-day culture period.

TABLE B-9. YIELD AND S CONCENTRATION IN *Cladophora glomerata* ISOLATED FROM LAKE ERIE GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

S added to culture soln.,* ppm	Ave. algae dry-wt yield,† mg/l	Ave. S conc. in algae, %
1.21	96	1.24
1.90	168	1.25
2.43	212	1.11
2.90	238	1.14
4.85	362	1.06
9.7	468	1.24
14.6	475	1.77
19.4	445	3.06

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 1.12 ppm Fe, 1/5 Johnson's trace elements, Na_2CO_3, Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

† Average of five replicates in each treatment; 18-day culture period.

TABLE B-10. YIELD AND S CONCENTRATION IN *Cladophora glomerata* ISOLATED FROM LAKE ONTARIO GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

S added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	Ave. S conc. in algae, %
1.21	183	0.44
1.90	206	0.98
2.43	196	0.55
2.90	221	1.09
4.85	431	0.85
9.7	659	1.32
14.6	641	2.00
19.4	653	>1.97

* The nutrient medium contained 23.4 ppm Ca, 16.4 ppm N, 2.8 ppm P, 18 ppm K, 7.5 ppm Mg, 1.12 ppm Fe, 1/5 Johnson's trace elements, Na_2CO_3, Na_2SiO_3, B_7 and C_{13} solutions, and vitamins B_1 and B_{12}.

† Average of five replicates in each treatment; 22-day culture period.

TABLE B-11. YIELD AND S CONCENTRATION IN THE GREEN ALGAE *Draparnaldia plumosa* GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

S added to culture soln.,* ppm	Ave. algae dry-wt. yield,† mg/l	Ave. S conc. in algae, %
0.12	173	.02
0.24	196	.06
0.49	306	.06
0.97	466	.12
1.94	702	.16
4.85	598	.25
9.70	827	.29
19.4	778	.69

* The nutrient medium contained 82 ppm N, 18 ppm K, 7 ppm P, 7.5 ppm Mg, 9.7 ppm Ca, 1.12 ppm Fe, 1/5 Johnson's trace elements, and Na_2CO_3 and Na_2SiO_3.

† Average of four replicates in each treatment.

TABLE B-12. YIELD AND S CONCENTRATION IN THE BLUE-GREEN ALGA *Nostoc muscorum* GROWN IN CULTURE MEDIUM DIFFERING IN S CONCENTRATION

S added to culture soln,* ppm	Ave. algae dry-wt. yield,† mg/l	Ave. S conc. in algae, %
0.12	115	0.11
0.24	192	0.12
0.49	284	0.13
0.97	249	0.06
1.94	243	0.13
4.85	258	0.26
9.70	245	0.28
19.4	230	0.34

* The nutrient medium contained 82 ppm N, 18 ppm K, 7 ppm P, 7.5 ppm Mg, 9.7 ppm Ca, 1.12 ppm Fe, 1/5 Johnson's trace elements and Na_2CO_3 and Na_2SiO_3.

† Average of four replicates in each treatment.